DISSERTATION ON
COMPARISON BETWEEN LIQUID BASED CYTOLOGY AND CONVENTIONAL CYTOPREPARATORY METHODS IN BODY CAVITY FLUIDS - A STUDY OF 100 CASES

Dissertation submitted to

TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
CHENNAI

for

MD (PATHOLOGY)

Under the guidance of

DR.S.MARY LILLY,M.D.,
Head of Department & Professor,
Department of Pathology,
Govt.stanley Medical College, Chennai.

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI-TAMILNADU

APRIL 2015

CERTIFICATE

This is to certify that this dissertation titled **"Comparison between liquid based cytology and conventional cytopreparatory methods in body cavity fluids"** is the original and bonafide work done by Dr.P.U.Swathy under the guidance of Dr.S.Mary Lilly, M.D., Head of the Department and Professor, Department of Pathology at the Government Stanley medical College & Hospital,Chennai-600 001, during the tenure of her course in M.D.Pathology from May 2012 to April 2015 held under the regulation of the Tamilnadu Dr.M.G.R. Medical University, Guindy, Chennai-600032

Prof.S.Mary Lilly,M.D
Professor and Head,
Department of Pathology
Government Stanley Medical College,
Chennai-600 001

Place:Chennai
Date:

Prof.A.L.Meenakshi Sundaram, M.D., D.A.,
Dean,
Government Stanley Medical College
Chennai-600 001

Place:Chennai
Date:

CERTIFICATE BY THE GUIDE

This is to certify that this dissertation titled Dissertation on **"Comparison between liquid based cytology and conventional cytopreparatory methods in body cavity fluids"** A Study of 100 Cases" is the original and bonafide work done by Dr.P.U.Swathy under my guidance and supervision at the Government Stanley Medical College & Hospital ,Chennai-600 001,during the tenure of her course in M.D.Pathology from May 2012-April 2015 held under the regulation of the Tamilnadu Dr.M.G.R. Medical University, Guindy, Chennai-600032.

Prof.S.Mary Lilly, MD.,
Head Of Department & Professor
Department of Pathology
Government Stanley Medical College
Chennai-600 001

Place:Chennai
Date: .9.2014

DECLARATION BY THE CANDIDATE

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Place:Chennai
Date: .09.2014

Signature by the candidate

Dr.P.U.Swathy

ACKNOWLEDGEMENT

I take this opportunity to express my heart felt gratitude to **Dr.S.Mary Lilly,M.D.,** my guide, Professor and Head of the Department of Pathology,Stanley Medical College,Chennai for her keen interest,constant encouragement, guidance and valuable suggestions throughout this study. Her constant motivation and drive were the key factors for the construction of this study .I am extremely grateful to her.

My heartfelt thanks to **Dr.P.Arunalatha, M.D.,** Professor of Pathology, Stanley Medical College, for the constant encouragement and guidance offered during the study.

My sincere thanks to **Dr.Nalli R.Sumitra Devi,** M.D., Professor of Pathology, Stanley Medical College for her immense help and support for the completion of this study.

I am extremely thankful to **Dr.K.Chandramouleeswari M.D.,** Professor of Pathology,Stanley Medical College for the constant encouragement and guidance offered by her during the study.

My heartfelt thanks to **Dr.K.Valarmathy M.D.,** Professor of Pathology, Stanley Medical College ,for the constant encouragement and guidance offered during the study.

My sincere thanks to **Dr.A.Jamila Rose, M.D.,** Professor of Pathology, Stanley Medical College,for her immense help and support for the completion of this study.

It gives me immense pleasure to thank **All my Assistant Professors** who has extended their valuable guidance and support during the study.

I am extremely thankful to **Dr.S.Y.Jagannathan M.D,** for his valuable support in compiling the statistics.

Last but not the least, I am grateful to all the **Faculty Members, My Colleagues and the Technical Staff Members** of the Department of Pathology, Stanley Medical College, **My Family Members and My Friends** for their constant support and encouragement during the period of study.

ABBREVATIONS

CEA	Carcinoembryonic antigen
CLL	Chronic Lymphoid Leukemia
CS	Conventional Smears
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetraacetic acid
FDA	Food and Drug Administration
LBC	Liquid Based Cytology
PAP	Papanicolaou

CONTENTS

ABSTRACT :

AIM :

To compare the morphology of cells by the two methods employed for processing of the fluids–"Conventional processing of fluids" and "Liquid based cytology technique" with regard to (a) cell yield (b) cell morphology (c) cell distribution (d) and background.

METHODOLOGY:

100 samples of body cavity fluids comprising of 33 pleural fluids, 56 peritoneal fluids and 11 urine were analysed. Smears were prepared using Liquid based cytology and conventional methods. All smears were stained by hematoxylin and eosin.

RESULTS:

Liquid based cytology showed better cell yield as compared to conventional smears in pleural, peritoneal fluids and urine. ($p<0.05$)Cell morphology was better preserved by Liquid based cytology than conventional smears in pleural, peritoneal fluids and urine. ($p<0.05$) Liquid based cytology showed more uniform cell distribution as compared to conventional smears in pleural ,peritoneal fluids and urine. These results showed a statistically significant difference between the two methods.($p<0.05$)Liquid based cytology lyywas not comparable to conventional smears in terms of background because the results

were not statistically significant. ($p>0.05$)This was true for pleural, peritoneal fluids and urine.

CONCLUSION:

Liquid based cytology was found superior to conventional smears in terms of cell yield, preservation of cell morphology and uniformity of cell distribution. Hence, Liquid based cytology can be preferred to conventional smears for cytologic examination of body cavity fluids. With regard to typing the characteristics of malignant effusions, more samples have to be analysed and a separate study is required.

Keywords: conventional method, liquid based cytology, pleural fluid, peritoneal fluid , urine

COMPARISON BETWEEN LIQUID BASED CYTOLOGY AND CONVENTIONAL CYTOPREPARATORY METHODS IN BODY CAVITY FLUIDS

INTRODUCTION

Exfoliative cytology is the study of spontaneously shed cells which line an organ or a cavity, from where these cells are removed by non-abrasive means.[1a] It comprises of study of cells from anatomic locations like effusions, CSF and synovial fluids as well as cells which are shed from urinary, respiratory and female genital tracts.

The most important features of exfoliative cytology are [1b]:

1) This technique is applicable to organs which are easily accessible.

2) The samples contain a wide variety of cells of various types obtained from different sources like inflammatory cells, macrophages, microorganisms, and material of extraneous origin.

3) Due to ongoing process of exfoliation , the cellular constituents are at times poorly preserved.

4) The most important advantage of exfoliative cytology is that multiple samples can be obtained from the same site.

The cells exfoliated in the fluids and washes can be concentrated by the process of centrifugation or the cells can be directly transferred on to the smears. This simple method of examination of cells by using light microscopy remains an important aspect till date, inspite of the tremendous progress in the development of sophisticated techniques like electron microscopy, chromosome analysis and DNA studies over the past several decades [2].

Exfoliative cytology aids in the diagnosis of cancer, inflammatory conditions like parasitic infestations and infections like bacteria, fungi or viruses. The diagnosis of cancer in pleural, pericardial or peritoneal fluids is of much importance for the patient as well as the attending physician or surgeon [1c].

There are several factors that complicate evaluation of the specimen which includes inflammation, blood, and the reactive mesothelium, which presents a continuum of morphologic changes, making distinction of malignancy difficult.

The introduction of liquid-based preparatory techniques allowed for enrichment of the cells in the preparations by reducing the inflammatory cells and blood [3]. The presentation of cells in a uniform layer allows the identification of malignant cells. The

liquid-based cytologic examination can decrease time and mental labor of the screening remarkably, because the cells are confined to a fixed area on the slide.

LBC techniques are currently applied to cytological samples from several tissues or fluids other than uterine cervix. They include endometrium[4-7], aspirates from breast[8-9] , thyroid tumors [10-11], ascites, pleural effusion[12], and urine[13-19]. Moreover, LBC technology is also suggested as an appropriate diagnostic method for metastatic tumors in cerebrospinal fluid [20] and other samples [21].

AIMS AND OBJECTIVES

1) Cytologic examination of pleural, peritoneal and urine received in our department during the period of June2012-June 2014.

2) To compare the morphology of cells by the two methods employed for processing of the fluids–"Conventional processing of fluids" and "Liquid based cytology technique" with regard to (a) cell yield (b) cell morphology (c) cell distribution (d) and background.

3) To draw the necessary conclusions with statistical analysis.

REVIEW OF LITERATURE:

HISTORICAL REVIEW OF

I. Exfoliative Cytology

The history of serous effusion cytology can be traced back to the 19th century. In 1838, "Donne", described the morphological appearance of cells in human colostrum which was the first report of exfoliative cytology. Bennet was credited as the first person who observed the tumor cells in effusion fluid in 1848 [22]. In 1860, Beale reported the identification of malignant cells in various body fluids. He found particles in sputum samples from a patient with malignant pharyngeal tumor[23]. The first mention of urine cytology for the purpose of diagnosis of bladder cancer was Sanders' report . He found neoplastic cells in urine in 1864 [24].Lucke and Klebs were apparently the first investigators who recognized the presence of malignant cells in an ascitic fluid in 1867. In 1882 Quincke gave the detailed descriptions of ovarian and lung cancer cells in serous effusions. However a century later , Keetel and Elkins established the idea of washing peritoneal cavity with normal saline for examination of spread of ovarian cancer[25].They published their results in 1956. In the year 1950, Crabbe published his work on the application of voided urine cytology for the surveillance of

workers employed in dyestuff industries in England. In the year 1960, Koss and his coworkers had several publications on the diagnostic value and limitations of voided urine specimens .They also introduced the concept of nonpapillary carcinoma in situ as the principal precursor lesion of invasive carcinoma of the bladder[26]. Papanicolau and Traut, in 1943 published the monograph. Since that time reports on effusion cytology have started to appear in the medical literature, and serous effusion cytology now is a routine diagnostic procedure worldwide.

II.Cytopreparatory Techniques

In 1685, Newton coined the term "centrifuge," which means to" flee from the center"[27] .In1965, Doré and Balfour first described a device for preparing cell spreads .In 1972,cytospin was used as a product name for the first time in commerce.In 2000, Cytocentrifuge was used to prepare thin-layer cervical cytology [28] . In 1896, first cell block was prepared in celloidin embedding medium. Centrifugation was introduced in 1901 into processing of cell block preparation to enhance cellularity. In 1956, Seal first introduced Millipore filters which was used for concentrating cancer cells suspended in large volumes of fluid.In 1964, Seal introduced Nuclepore filters [29].In 1959, bacterial agar was first introduced for

the preparation of cell blocks . In 2007, Automated cell block system was introduced into commerce. In 1927, Dr.Papanicolaou, introduced the PAP smear for cervical screening. This simple preparatory technique has saved lives of million women.First significant change occurred in the cell preparation in mid 1990's since 70 years of discovery of Pap smear. An innovative new generation of products were introduced to enhance the sample processing, a technology that helped the laboratories to "clean the specimen". It was known as liquid based cytology (LBC). This term "liquid-based preparation" was introduced in 1998 [30]. U.S. Food and Drug Administration (FDA) approved Thinprep™ technology as an alternative method to conventional method done for cervicovaginal smears in 1996.This was followed by approval of the AutocytePrep™ , which is now known as Surepath™, BD Tripath, Burlington, NC. Surepath was approved 3 years after Thinprep in 1999. Liqui Prep is yet another liquid based cytology technique which is being manufactured by LGM International Inc., located in Florida, USA. Liqui Prep™ has been approved by the US FDA, CE Mark, Thai FDA in 2004.It has been available worldwide since then. The latest technology in Liquid based Cytology is Monoprep™ which has obtained its approval in 2006[31a].

EFFUSION CYTOLOGY

Accumulation of excess amount of fluids in the serous cavities of body is known as effusion. Depending upon the site of localization, they are classified as pleural, pericardial and peritoneal effusions.

TYPES OF EFFUSIONS

Mesothelial cells are affected by many stimuli like inflammation, cirrhosis, congestive cardiac failure and neoplastic process. The damaged mesothelial cells are replaced by mesenchymal cells in the underlying stroma.

TRANSUDATES

- Due to increased venous pressure
- Capillary walls are intact
- Low protein (<3g/dl)[32]
- Low specific gravity(<1.015)[32]
- Causes – congestive cardiac failure, cirrhosis, renal failure and hypoproteinemia
- Low cellular content – mesothelial cells, macrophages and occasional neutrophils or lymphocytes

EXUDATES

- Damage to capillary walls
- High protein(>3g/dl)[32]
- High specific gravity (>1.015)[32]
- Causes are: infections and neoplasm
- High cellular contents: inflammatory cells in inflammation and neoplastic cells in malignancy.

DEPENDING UPON THE PATHOGENESIS

1.Hydrostatic

- Due to imbalance between the intravascular pressure and oncotic pressure.
- These type of effusions contain benign mesothelial cells, few inflammatory cells and sometimes blood if it is a traumatic tap.
- Causes are Cardiac failure (increased hydrostatic pressure), liver failure (decreased oncotic pressure), renal failure, myxedema, peritoneal dialysis, Meig's syndrome and exudative enteropathy.

2. Infectious

- Due to direct invasion of the organisms or as a result of byproduct of inflammation.
- Inflammatory cells and mesothelial cells are usually seen.
- Mesothelial cells show continuum of changes.
- The kind of inflammatory cells give information about the causative organism.eg; lymphocytes indicate tuberculosis.
- Causes like bacterial and viral infections.

3. Non-Infectious

- Due to autoimmune or due to response to stimuli.
- Inflammatory cells are variable and the mesothelial cells show a spectrum of atypia.
- Causes are Rheumatoid arthritis, Systemic Lupus erythematosus, radiotherapy and tissue necrosis.

4.Malignant

- The cytopathologist should be aware of the details of the past and present disease state for better reporting.

- The cytological evaluation reveals a uniform population of cells in case of primary mesothelioma and a second population of cells along with benign mesothelial cells in case of metastatic lesions.The common primaries are tabulated as follows: (table no:1)

Table No-1: Common sites of primary for malignant pleural and peritoneal effusions

Type of Effusion	Common Sites of Primary in Men	Common Sites of Primary in Women
Pleural	Gastrointestinal tract[33]	Breast followed by lung and ovary[33]
Peritoneal	Gastrointestinal tract followed by pancreas and lung[33]	Gastrointestinal followed by pancreas[33]

Metastatic tumours involving the pericardial tissue can cause pericardial effusions.

SAMPLING TECHNIQUES OF SEROUS EFFUSIONS

Pleural Fluid

It can be obtained by thoracocentesis and pleural lavage.

Thoracocentesis: Once pleural effusion is diagnosed in a patient, the first step is finding the cause of the effusion.

Indications of thoracocentesis

- If the pleural fluid is more than 10mm in lateral decubitus position as evidenced by X-ray, which is new in onset and without any known etiology.

- If the pleural effusion is persistent for 3 consecutive days.

PROCEDURE

A needle is inserted through the sixth, seventh, or eighth intercostal space in the midaxillary line and entered into the pleural space. If the etiology of the effusion is due to cardiac failure , examination of single sample is sufficient.

The pleural lavage is used for staging of lung cancer and oesophageal cancers[34-38]The cytologic examination helps in identifying the cause of effusion as well as prognosis of the disease.[39]

Peritoneal Fluid

There are various ways of obtaining peritoneal fluids like ascitic fluid, peritoneal washings and peritoneal dialysis fluid. For staging of tumours, sample should be collected immediately after entering the peritoneal cavity because tumour cells can spill into the peritoneal cavity during the procedure of exploratory

laparotomy and removal of primary tumour. Any sample is designated as “peritoneal fluid” if we see pre-existing spontaneous fluid in the pelvis and as “Ascites” if the fluid is excessive. The peritoneal washing is obtained by instilling 100 ml of saline or balanced salt solution into the peritoneal cavity, agitated, aspirated and cytological evaluation is done. Peritoneal dialysate samples are mostly sent for eosinophil count to ruleout eosinophilic effusions caused by irritation of the peritoneal dialysis catheters. When the cause of ascites or chronic liver disease, examination of single sample is enough.[40] There are few differences between ascitic fluid and peritoneal washings which are tabulated in Table No:2.

Indications For Peritoneal Washings

1) Staging of gynaecological malignancies like ovary , fallopian tube and endometrium

2) Ruling out occult cancer

3) Response to previous treatment

4) Staging non-gynaecological malignancies like pancreas and stomach.

Table No-2: Difference between ascitic fluid and peritoneal washings

Ascitic fluid	Peritoneal washings
Collected by spontaneous exfoliation	Cells are mechanically stripped from the underlying connective tissue
Cells arranged in three dimensional groups	Two dimensional groups
Cells usually round in shape	Cells are usually flat mesothelial cells

Advantage of ascitic fluid is that no trauma is inflicted to the mesothelial surfaces and can be easily obtained. Peritoneal washings is better than ascitic fluid for staging of tumours as it reflects the natural biology of patients's tumour.

Disadvantage of peritoneal dialysis fluid is that the cells may show cytological atypia which may be misinterpreted as malignancy[41].

Peritoneal washings are used in the prognostication of various malignancies.Prognosis based on the presence of malignant cells in the peritoneal fluid depends on the nature of the primary tumour which is tabulated below (table no:3)

Table No-3: Prognostic value of positive peritoneal washings in various malignancies

Primary Tumour	Effect on Prognosis
Ovarian	To continue therapy[42]
Endometrial	Indicate adnexal involvement[43,44]
Cervix	Advanced disease and necessitates endocavitary chemotherapy[45]
Stomach	Peritoneal recurrence and poor prognosis[46]
Pancreas	Advanced disease[47]

URINE CYTOLOGY

Urine cytology is a very effective tool in the diagnosis of high grade neoplasms of the bladder[48]. The diagnosis of low-grade neoplasms are much difficult due to the similar cytomorphology as that of normal exfoliated urothelial cells and to those seen with calculi, inflammation and instrumentation. It is important to audit periodically the appropriateness of clinical requests for urine cytology and adherence to agreed guidelines[49].

INDICATIONS FOR CYTOLOGICAL EXAMINATION OF URINE

1) Evaluation of hematuria in suspected cases of malignancies of urinary tract.

2) To follow up of cases of carcinoma in situ and invasive bladder carcinoma

3) Assessment of glomerular damage in renal diseases.

Urine samples are obtained by different ways. They are voided urine, catheter sample, bladder washings and ileal conduit samples.

VOIDED URINE

Voided urine can be

- Randomly voided
- Voided after hydration
- Collected after 24 hours
- Collection from intestinal conduit

It is the least expensive method. First or last part of voided urine is rich in cells. Early morning samples are not preferred because the cells are degenerated due to overnight stagnation of urine in the bladder. Hence the second sample is required. Most useful specimen is voided urine. Atleast 3 voided samples collected

in 2 weeks is essential for identification of malignant cells in urine samples. For cytological testing, either a mid- morning or random specimen is recommended .If there is significant delay, it is either refrigerated or fixed with 50% ethanol or Saccomanno's fixative [48]

Advantage

1) Diagnosis of high grade tumours

2) Diagnosis of human polyomavirus infection

3) Follow up of locally treated tumour

Disadvantage

1) The results are not consistent hence atleast three samples are required.

2) Contamination with cells from female genital tract

CATHETERISED URINE

In the catheter sample, since urine is collected in the bag at room temperature for long time, the cells are degenerated. Only enough lubricant should be added to the catheter. If there is too much of lubricant, it will accumulate in the samples and obscure the cellular details.

Advantage

It has less contamination with cells of female genital tract

Disadvantage

Same as voided urine.

ILEAL CONDUIT SAMPLES

Ileal conduit samples are used to follow-up the patients with carcinoma bladder since they have high risk to develop carcinoma of ureters and kidneys. They should be collected as fresh as possible.

Disadvantage of this method is that the morphology of the shed urothelial cells may mimic neoplasia hence posing diagnostic problems.

BLADDER WASHINGS

Bladder washings are done by urologist by instilling 50- 100 ml of balanced salt solution via a large volume syringe connected to the cystoscopy port. The fluid is withdrawn and reinjected with some force to dislodge the epithelial cells and then is examined.The procedure should be done before any manipulation of bladder like biopsy. Advantage of this method is high cellular yield. It is the most useful technique for DNA measurements.

SPECIMEN COLLECTION

BODY CAVITY FLUIDS (Pleural,pericardial and peritoneal fluids):

- 50-100 ml of fluid should be sent in clean dry container
- Formalin /Alcohol should not be added.
- Fluid is processed as early as possible
- If there is any delay in delivering the sample, it should be refrigerated at 4 degree Celsius.

URINE (including voided, catheter ,urethral washings and ileal conduit samples):

1) 20 to 50 ml of urine collected in clean dry container.
2) Second voided sample is preferred.
3) If there is any delay, store at 4degree Celsius.

TRANSPORTING OF FLUIDS

Anticoagulants are added to the fluids. Anticoagulants like heparin, acid citrate and dextrose ,disodium EDTA and oxalate are used. 3-5units of heparin are added to 1ml of fluid.[50]Heparin is the commonly used fluid anticoagulant in cytology.

HEPARIN

Advantage

Helps for the uniform suspension of the body cavity fluids and preservation of cellular morphology.

Disadvantage

It interferes with quality of Romanowsky stains because it causes background staining[51].

ACID CITRATE DEXTROSE & DISODIUM EDTA[48]

Advantage

Preservation of cellular morphology and absence of background staining.

If there is any delay in transporting, specimens should be refrigerated or fixative has to be added.

FIXATIVES FOR EXFOLIATIVE CYTOLOGY:

Fixatives For Body Cavity Fluids

1.Ethanol - 50% ethanol is the universally used fixative for fluids. If more than 50% concentration is used, it will cause hardening of the sediment and smearing will be difficult , especially when there is a delay of more than 1 hour for processing. For similar reasons, ether and acetone are not used as fixatives for

fluid specimens. Equal volume of fixative as that of the fluid specimen should be added.

2. Saccomanno's fixative comprises of 50% alcohol and 2% Carbowax . Carbowax infiltrates the submicroscopic spaces occupying them thereby preventing cell collapse. Hence it provides protection from air drying effect on the cells. There is good adherence of the cells to glass slides as a result of air drying.

3. Shandon Mucolexx is a commercial fixative. It liquefies the mucus. It is used for mucoid and fluid specimens. It is composed of polyethylene glycol, methanol, buffering agents, and aromatics. An equal volume of undiluted Shandon Mucolexx is added to the sample.

4.Many commercial preservatives used in automated cytology systems have practical application for routine cytological examination. In 1997, Weidmann et al tested CytoRich Red. It consists of buffering agents, emulsifiers, formaldehyde, and alcohol .It is developed for use in TriPath PREP as a preservative.[1d]

GROSS EXAMINATION OF FLUIDS

Volume and gross appearance of the specimen should be documented as soon as the fluid specimen is received since, gross examination of fluid will aid in the diagnosis. Physical features like volume, colour, clarity, opalescence, odour and viscosity should be assessed. 1. Volume which gives an idea about the cytopreparatory techniques 2. Colour of the fluids will guide diagnosis. Most of the malignant effusions are grossly blood stained but only proportion (46%) of them are positive for malignant cen lls.[52]

Etiology of few diseases can be obtained from the colour of body cavity fluids. They are discussed as follows.(table no:4)

Table No.4: Diagnosis based on morphological appearance of fluids

Heavy white and flocculent sediments with Lime or pineapple juice colour supernatant	Rheumatoid serositis
Milky white with creamy top layer	Chyle
Yellow and turbid shimmers on agitation	Cholesterol crystals
High viscosity	Diffuse malignant mesothelioma, metastasis from Wilm's tumour, pseudomyxomatous peritonei
Chocolate brown	Melanoma cells
Light brown	Chronic haemorrhage due to hemosiderophages
Brown-orange or green	Jaundice or leakage of bile

PRESERVATION OF FLUID SPECIMENS PRIOR TO PROCESSING:

Specimens with high protein content:

Pleural, peritoneal and pericardial fluids can be preserved by refrigeration for 24-48 hours. The high protein content of fluids help in preserving cell morphology by acting as a tissue culture medium.

Specimens with Low Mucus or Protein Content

Fluids like cerebrospinal fluid or urine can be preserved by refrigeration for 1-2 hours. Even if refrigerated, they cannot be preserved for more than 1-2 hours. These fluids contain enzymatic agents which cause cellular destruction. Refrigeration inhibits bacterial growth but does not protect the cells.

GENERAL PRINCIPLES OF PROCESSING OF FLUIDS:

Routine Processing

The sample is stirred briskly for dispersing the cells. A representative sample is taken and centrifuged at 2500rpm for 5 minutes. If the quantity of the fluid is too little an equal volume of normal saline is added before centrifugation. Place one or two drops of sediment on the slide and allow it to evenly spread by placing another slide over it.[48]

For Sparsely Cellular Fluid

Cytocentrifugation helps in concentrating the cells. Fluid to be concentrated is first centrifuged at 2000rpm for 10 minutes. Majority of the supernatant is discarded, with few drops left in the bottom of the centrifuge tube. This portion of fluid is stirred well and 2-5 drops are used for cytocentrifugation. Cytocentrifuge, spins the samples at 2000rpm for 2 minutes and sediments the cells directly on the slides. The fluid medium is absorbed by the filter card. Disadvantage of this technique is distortion of cellular morphology because of the drying artefacts.

Hemorrhagic Fluids

Carnoy's fixative or glacial acetic acid is used to lyse the RBC's.

CYTOPREPARATORY METHODS

1) Direct smears
2) Conventional centrifugation
3) Cytocentrifugation
4) Membrane filtration
5) Cell block
6) Liquid based techniques or thin layer technology

DIRECT SMEARS

Direct smears are prepared from fresh unfixed specimens. It is done by placing a drop of specimen directly on the slide and

smearing it. Specimen can either be before or after concentration as sediment. They are three types of direct smears.

1. Stained wet films 2.Wet fixed smears 3.Air dried smears

STAINED WET FILMS

Has been advocated by Bernard Naylor. It is prepared by placing a drop of sediment of unfixed specimen on the center of the slide and a drop of toluidine blue is added next to the drop of sediment. With the corner of coverslip both the drops are mixed and examined immediately and discarded after evaluation. They are cytologic equivalents of frozen sections in histopathology.

Advantages

1) Immediate diagnosis (within 10-15minutes of specimen arrival)

2) Certain features not seen in permanent smears are seen, like cholesterol crystals, Charcot-Leyden crystals, hematoidin crystals, psammoma bodies and detached ciliary tufts.[53]

3) Triage of fluid for microbiology, immunocytochemistry, cell biology, flow cytometry and cytogenetics.

4) They are used to identify superpositive effusions i.e) those effusions flooding with cancer cells. Identification of these smears are useful to prevent cross-contamination with other specimens.

5) They aid in identifying unusual or interesting cytologic specimens, so that additional smears for cell block preparation can be made.

WET FIXED SMEARS:

Smears immersed in fixative prior to drying are called wet fixed smears. Fixatives used are 95% ethanol,95%methanol, 95% isopropanol and carbowax. These smears are used for Papanicoloau stains. Disadvantage of this method is that it is not used for Romanowsky stains. It is more useful for studying nuclear detail, nucleoli, squamous differentiation &keratinization , oncocytes, psammoma bodies and lymphoid cells (nuclear outline, chromatin pattern and nucleoli).

AIR FIXED SMEAR

A smear which is dried completely by gentle moving or with a hair dryer is called air fixed smear[54].It is used for Romanowsky stains. If the smear needs to be used for Papanicoloau staining, they should be used after saline- rehydration followed by fixation with 95%ethanol or 95% ethanol with 5% acetic acid[55]. It is more useful for studying cytoplasmic details , stromal component , mucin , colloid ,secretory granules (prostate), bare bipolar nuclei (benign

breast) and lymphoid cells (lymphoglandular bodies, cytoplasmic basophilia and lipid vacuoles).

CONVENTIONAL CENTRIFUGATION

This method is used in our laboratory for all types of body fluids. Conventional centrifugation is a method in which constant centrifugal force is applied for a constant time. Swinging bucket centrifuge is used. It is the most common method used for concentration of the specimen. For urine, low centrifugal forces are applied, whereas high centrifugal forces are used for proteinaceous fluids. Packed sediment is formed at the bottom of the centrifuge tube. The supernatant can be removed entirely or little volume is left behind for further processing. Graduated conical or nipple test tube can be used which will enable proper visualization of the nature of the sediment and aids in the measurement of the volume. The assessment of the nature of the specimen is important for deciding the need for lysing the red blood cells and mucus. The volume of specimen is used to determine the subsequent dilution of the specimen. The sediment is inverted on the gauze and allowed to stand until it is dry.

Close observation of cell button is necessary to assess its loss. If there is excessive blood or protein, it will affect the staining

quality. On centrifugation of bloody samples, a buffy coat is formed. The buffy coat is rich in white blood cells and mesothelial cells. The supernatant can be removed with Pasteur pipette and buffy coat smear can be made which is rich in cellularity.

CYTOCENTRIFUGATION

Principle

In conventional centrifugation, cells are distorted while depositing and smearing. During cytocentrifugation, cells are sedimented directly onto a vertical slide while the suspension medium is absorbed by the surrounding absorbent paper ring.

There are many cytocentrifuges which are available commercially

1) Shandon cytocentrifuge (See Fig.1)
2) Wescer Cytopro
3) Hettich cytocentrifuge

Uses of cytocentrifuge:

1) Nongynecological samples especially hypocellular fluids
2) Microbiology
3) Bone marrow and peripheral smears where the cellularity is low
4) Virology
5) Research on molecular studies.

Fig1.Shandon cytocentrifuge

Difference between conventional centrifugation and cytocentrifugation are tabulated below : (see table no:5)

Table No-5: Differences between conventional and cytocentrifugation

Conventional centrifugation	**Cytocentrifugation**
Constant centrifugal force for constant time	Controlled centrifugation at right angles to the slide.
Forms sediment.	Does not form sediment.
Smear made from sediment	Produces a cell monolayer directly on the slide.
Cell distortion more.	Cell distortion less.

MEMBRANE FILTRATION

The main principle is to flatten cells to enhance chromatin visualization.

There are two types of membrane filters (see table no:6)

1) Cellulose eg) Millipore and Gelman
2) Polycarbonate.eg) Nucleopore

Table no.6: Differences between Millipore and Nucleopore

Millipore	Nucleopore
White and opaque until cleared with xylene.	Colorless.
140microns thick . Refractive index same as that of the mounting medium.	10microns thick. Different from the mounting medium and birefrigrent.

Advantage

Cell recovery is good even in sparsely cellular samples.

Disadvantage

1) Reaction of the chemicals with the filters.

2) Clogging of the filters by blood, mucus or urinary salts.

Advancement in this technique is the Cytotek MonoPrep Manual Filtration System.

CELL BLOCKS

Processing common to all types of cell block preparation are

- ❖ Cells in suspension are centrifuged to form a cell concentrate or pellet.
- ❖ Cells are fixed.
- ❖ The cells are embedded in situ so that they can be removed en bloc from the centrifuge tube.
- ❖ Processed like tissue processing.

TYPES OF THE CELL BLOCK PREPARATIONS

1) Histogel (Steven et al)[56]

2) Gelatin embedding (Nithyananda et al)[57]

3) Agar embedding method

4) Plasma-thrombin method

5) Celloidoin

6) Cell block preparation from scraped material from cytology smears

7) Cell block preparation from Millipore filters

Advantage

Multiple sections can be made. Routine hematoxylin and eosin and special stains can be done. Immunohistochemistry can be performed in cell blocks helping in diagnosis. Cell blocks can be used for retrospective studies too.

LIQUID BASED CYTOLOGY

Liquid based cytology is a methodology used to rinse samples in liquid preservative, which are then transported to the laboratory, where the sample is partially homogenized and a subsample, thin-layer preparation is made. Monolayer provides a visual image of the process, but most preparations are not truely monolayers. Thin layer is more accurate than monolayer, but some conventional smears can be thin layer too. Liquid based cytology has been developed to increase the sensitivity and specificity of cytologic examinations so that it can be used as a screening as well as a diagnostic modality. Many proprietary systems are now available which are based on manufacturer specific manual, semi-automated or fully automated protocols. The basic principle of these systems are broadly similar, keeping in mind the main aim of preparing a homogeneous and clearer sample which occupies only smaller portion of the slide. This helps in easy and quick screening along

with decrease in the unsatisfactory specimens. This technology is being used widely for both gynaecological and non-gynaecological specimens.

EVOLUTION OF LIQUID BASED CYTOLOGY

Two types of LBC are in use. The First generation LBC & Second generation LBC.

First Generation Liquid Based Cytology

Thin Prep and Surepath are used worldwide. Both have also been used for nongynecological cytology(Yukihiro Kobayashi et al 2011)[58].

Second Generation Liquid Based Cytology

The second generation of liquid based cytology has evolved to reduce the instrumentation and to make these techniques cost effective.

The new second generation liquid based cytology are

- Cell solution 120 (Synermed)
- Liquiprep (LGM)
- PapSpin (Shandon)
- Cytoscreen (Serosa)

- Turbitec (Labonord)
- Cell slide (Menarini)
- MonoPrep Pap (MPPT)
- MonPrep2 (MP)

BASIC PRINCIPLE OF LIQUID BASED CYTOLOGY

Liquid based cytology works on either one of the two principles with a common goal. The goal being examination of cells of interest removing the unnecessary ones. The two principles are Precipitation and Filtration. (Ji Hae Koo et al)[59]

1) Precipitation- eg.MonoPrep , Thinprep and Cellprep
2) Filtration – eg.Surepath

THIN PREP

The entire procedure uses a disposable plastic tube lined by filter.

Specimen is collected in methanol- based medium and centrifuged. The cell pellet which is obtained is transferred to a methanol-based preservative. There are two commonly used preservatives in ThinPrep technique -

CytoLyt® Solution and PreservCyt® Solution.

CYTOLYT® SOLUTION

- ❖ Methanol-based, buffered preservative solution
- ❖ Lyses red blood cells
- ❖ Prevents protein precipitation
- ❖ Dissolves mucus
- ❖ Preserves morphology for 8 days at room temperature
- ❖ Intended as transport medium
- ❖ Used in specimen preparation prior to processing

PRESERVCYT® SOLUTION.

- ❖ Methanol based, buffered solution
- ❖ Specimens must be added to PreservCyt Solution prior to processing
- ❖ PreservCyt Solution cannot be substituted with any other reagents
- ❖ Cells in PreservCyt Solution are preserved for up to 3 weeks in a temperature range between 4-37 degree Celsius.

3 main principles in the cell preparation by Thin Prep are

1) Cell dispersion
2) Cell collection
3) Cell transfer

Cell dispersion (See Fig. 2a)

The machine introduces the disposable filter tube with a polycarbonate filter into vial which contains the cell suspension and agitates it. The size of the pore in the filter is 5.5 microns. This will cause dispersion of the mucus and cell clumps.

Cell collection: (See Fig. 2b)

Small vacuum pulse is applied which will drain the fluid into the tube through the filter. As a result, a layer of cellular material is deposited. But some amount of blood inflammatory cells and cell debris can pass through the filter. The fluid flow is monitored in order to optimize the cell capture.

Cell transfer: (see fig. 2c)

The filter is inverted and pressed gently against an electrostatically charged slide.

Fig2a.
Cell dispersion

Fig2b.
Cell collection

Fig2c.
Cell transfer

The slide is immersed into the fixative immediately. Then the staining is either done manually or automated. This produces a relatively thin, monolayer-type preparation (Tarik et al)[60].

Whole procedure takes about 30minutes. Cell deposition area is 2cm.

TP-2000 is a semiautomated device which can handle one specimen at a time. TP-5000 is a fully automated device which handles specimens in batches of 20.

Numerous studies have been done to evaluate the efficacy of Thinprep™ preparations on the body cavity fluids .A study

conducted by Bong et al[61] on urine specimens showed that Thinprep preparations has certain advantages like better preservation, cellularity and clear background.

A study conducted on cerebrospinal fluid samples by Sioutopoulou et al[20] showed ThinPrep technology provided better preservation of cytomorphologic features, high cellularity per slide and clear background.

Another study done by Elsheikh et al compared Thinprep with cytocentrifuge techniques using varied specimens like body cavity fluids and urine and demonstrated that ,Thin Prep showed more uniform distribution of cells, superior nuclear chromatin morphology and less cellular overlapping and background debris[60].

Alwahaibi et al[62] conducted a study using peritoneal and pleural fluids comparing ThinPrep with conventional techniques and drew few conclusions 1.Thinprep showed monolayer architecture with minimal overlapping, better cytomorphology and lesser slide evaluation time as compared to conventional techniques 2.thinprep is more expensive than conventional techniques 3.Conventional smears are more cellular than Thinprep smears.

Another study conducted by Babloyan et al[63] on peritoneal washings and ascitic fluids observed that ThinPrep method showed better cytological details, significant improvement in the diagnostic accuracy of the cytological diagnosis of ovarian cancer, reduces the screening time of the slides and permitted the valuable application of current techniques of static DNA cytometry.

One another study done by Argon et al comparing liquid based cytology and cytocentrifugation on cerebrospinal fluids showed that despite slight decrease in suspicious diagnosis, there was an increase in malignant and benign diagnoses with the LBC method in comparison to the centrifugation method.

SURE PATH™(TRIPATH IMAGING INC)

The sample is collected in an ethanol based fixative and sent to the laboratory. In the laboratory , the vial is vortexed in order to disperse the cells. Then CyRinge, a Sure Path proprietary device inserted into the collection vial to disintegrate the larger cell fragments. The Cyringe later is then inserted into centrifuge tube of 15 ml capacity which is filled with 4 ml of SurePath Density gradient fluid. The samples are made to flow through the drainage tube onto the top of the density gradient fluid; Specimen is transferred to a sedimentation tube ,centrifuged and a cell pellet is

formed. The cell pellet is resuspended and the sedimentation process is repeated again. The procedure is completed using the PrepStain™ slide processor, in which a robotic arm transfers the fluid to a settling chamber, which settles the cell pellet on the top of a modified poly- l -lysine-coated glass slide. Robotic arm then stains the slides on the PrepStain™. The main principle of cell enrichment in this technique is by the density gradient centrifugation.

Density Gradient Centrifugation

Density gradient fluid is composed of concentrated solutions of sugars and other substances formulated in such a way that when an aliquot of sample is added on the top of this fluid and centrifuged, different cell types are separated into different layers based on their specific gravity.

Generally the epithelial cells have a different specific gravity as compared to the non-epithelial cells , hence they have a tendency to concentrate in the form of a layer.

This step not only causes cell enrichment but also removes blood and other contaminant debris. Supernatant fluid is removed and the formed cell pellet is resuspended and centrifuged once

again. A robotic arm transfers the aliquot of cell pellet to a settling chamber where the cells are allowed to sediment under gravity thereby producing a thin layer on poly-l-lysine coated slide.The machine stains the slide automatically.

Entire procedure takes 60 minutes. Circular deposit area is 1.3 cm in diameter.

The SurePath ^{TM} liquid-based cytology (SP-LBC) system has become widely utilised as a technique for the purpose of collection and preparation of gynecological specimens.There are few differences betwee Thinprep and Surepath which are discussed in table no:7

Several studies conducted on this techniques showed that the Sure Path ^{TM} method has an improved diagnostic sensitivity than conventional preparation methods for gynecological specimens[64-66]. Several methods using liquid-based thin-layer preparations for non-gynecological cytology specimens have shown improved diagnostic accuracy, and the liquid based methods are being used increasingly. However, reported pathological studies of the use of LBC techniques in body cavity fluids are limited[67,68].

They are only few studies comparing the performances of SP in fluids. One such study conducted by Zardawi and Duncan[68] found that, Cytospin method had longer preparation time but shorter screening time than the Surepath. The number of diagnostic cells was higher in the Cytospin method. Fixation quality and staining clarity were better in the Cytospin method. Qualitative assessment of cell arrangements, cell and nuclear size and shape, nuclear/ cytoplasmic ratio and nuclear membrane irregularity showed no significant differences between the two methods. Cellular details and nuclear chromatin patterns were clearer and better preserved in the Cytospin method, but Sure Path method showed less blood and inflammatory cells and debris.

Another study conducted by Zendehrokh et al[67] comparing Surepath with cytospin made the following observations i) SurePath preparation decreased the number of insufficient samples and atypical cases ii) SurePath reduced obscuring blood and salt crystals but left enough background material to provide diagnostic clues.

Table No.7: Comparison Between Thin Prep and Sure Path

	Thin Prep	**Sure Path**
Cost	More expensive	Less expensive
Slide preparation	Fully automated	Partially automated
Cell deposition	20mm diameter	13mm diameter
Cellularity	Lower	Higher
Cell distribution	Uniform, 1 plane of focus	Uniform , many planes of focus
Cell morphology	Less well preserved	Better preserved
Extracellular material Quantity Appearance	 Reduced Altered	 Less reduced Less altered

LIQUIPREP

First generation LBC technology posed two challenges.

1) Requirement of automated instrument

2) These devices are designed around vacuums, filters and plastic disposables.

This resulted in increase in complexity of the device and also the cost of each test. Even though these technologies are much superior to the conventional preparations, adoption was difficult due to its high costs , and for many laboratories, the instrument

didn’t meet the laboratory needs.So, a new second-generation LBC technology has evolved known as Liquiprep.

Liquiprep has 3 components

1) Specimen preservative

2) Specimen cleaner

3) Cell base reagent

Specimen Preservative

These preservatives have many advantages

- ❖ Helpful in follow-up testing since they can be used for molecular and immunochemistry techniques.
- ❖ Lyses red blood cells in bloody specimens and digests mucus.
- ❖ Suppresses bacterial growth hence acts as an antibacterial agent during transportation of specimens.
- ❖ Specimens are stable in the preservatives for about 90 days.
- ❖ Can be transported and stored at room temperature.
- ❖ Preservation of cells without disruption of classic cell morphology.

- Formulations are non-hazardous hence need for safe disposal is eliminated and the shipping costs are reduced.

Specimen Cleaner

- It works on the principle of gradient density technology.
- Red blood cells and mucus which are lighter gets trapped at the top of the cleaner solution.
- Denser cells i.e clinically relevant cells will travel through the cleaning solution and results in the formation of a compact pellet in the bottom of the centrifuge tube.
- For specimens with dense blood and mucus, Liqui-prep Lytic Reagent
- is used which provides additional cleaning power.
- Centrifugal forces allow all cells to settle in the form of pellet. The whole cell clones descend intact.
- Since the filters are not present, there is no clogging of cells.
- The entire specimen is processed.

Cell Base Reagent

Main purpose of this reagent is the suspension of cells in monolayer sheets.

It is prepared using agarose, poly ethylene glycol, absolute alcohol and poly-l-lysine.

This is an adhesive with unique features

- Provides good adhesion to even standard laboratory slides.
- Eliminates the need for electrostatically charged slides.
- Since cell concentration can be controlled using this solution, this method can be used for specimens with variable cellularity
- Is compatible with broad range of pathology stains.
- Is permeable to molecular methods and immunocytochemistry.
- For those specimens with small cell pellets, cells can be concentrated to reduce reading time.

Advantages of Liquiprep

- No capital investment is needed
- Cost per test is less.
- Cheaper to ship.
- Environmentally friendly, eliminating the need for safe disposal.
- Molecular and immuno friendly.
- Clean background with better preservation of cells compared to CS.
- Area of examination of slide is reduced and thereby decreases the duration of screening.

Hence this technology can be employed in developing countries[69].

Procedure

First cleaning solution is added to the labelled centrifuge tube. Specimen is mixed well and then poured into the centrifuge tube. Centrifuge at 1000g for 10minutes.Cell pellet is formed after

pouring off the supernatant .Add cell base in the ratio of 1:3 based on the nature of the specimen. The cell base is suspended well by vortexing 50 microliters of the mixture is pipetted out and smeared in a circular manner on the slide. The slides are dried at room temperature and then stained. (Jongkolnee Settakorn et al 2008)[70]

There are various studies showing the efficacy of Liquiprep system in cervicovaginal smears[71,72]. There are limited literature for Liquiprep in body fluids. One study conducted by Gyeongsin Park et al[73] on cerebrospinal fluids showed that LP is superior to CS in view of cytopreservability and for rendering a definite diagnosis.

Another study conducted by Norimatsu et al[74] compared two methodologies of Liquid based cytology i.e Liquiprep and Surepath on urine samples. According to their study, preservation of cell morphology was comparable between two methods.

CELL PREP PLUS

This is another fully automated system which is recently approved by FDA. In CellprepPlus® , the transfer of cells from a preservation liquid to a slide is done in two steps:

- Once the liquid preservative bottle is placed into the CellprepPlus® device, it filters the cells with its own pressure.

- Using air pressure, blows cells from the filter to the slide .As a result, the celoidls are transferred to a 20 mm circular area in a thin layer.

ADVANTAGES OF CELL PREP PLUS

1) Automated capping system: The need for opening the cap during each process is eliminated.

2) Automated cell transfer system.

3) Automated Filter supply system

50 filters are loaded at once and the new filters are automatically replaced during the beginning of each test.

Rapid. 30secs for 1 slide

There are studies regarding the performance of Cell prep plus in body cavity fluids.

A study conducted by Seung et al[75] showed that CellprepPlus® LBC gave increased cellularity, more cleaner background, and superior maintenance of cell morphology as compared to conventional techniques . LBC is more cost-effective than CS because it reduces the need for repeated examinations. Furthermore, residual samples could be used to process multiple slides for ancillary tests for immunocytochemistry and molecular tests.

Another study conducted by Koo et al[59] noted that CellprepPlus® LBC for body fluid showed increased sensitivity and negative predictive values ,making it suitable in the screening of body fluids.

CYTOSCREEN:[31b]

This is a manual process. It is a much simpler technique. It has additional step to check the cellularity of the sample for its standardisation. For this, it relies on the concept of

photometry prior to centrifugation on slide. This method uses centrifugation to produce a layer of cells.

LABONARD EASY PREP

Another manual process. Here the aliquot of sample is loaded into a separation chamber attached to glass slides which contains absorbent paper. The cells settle in a thin layer and preparation is stained using normal laboratory procedures.

This is a simple liquid-based technology and it does not require a major capital expense.

The system consists of

1) Preservative with mucolytic action

2) Syringe

3) Housing assembly with proprietary filter

4) Fixative for the preparation of slides.

Specimens can be collected either with the preservative or the preservative can be added to fresh samples in the laboratory. The procedure begins with attaching the syringe, housing the filter to the collection vial and the plunger is pulled back until it locks. After the fluid stops flowing into the chamber , which occurs only

when the filter becomes covered with cells, the filter is placed, cell side down on a glass slide. The fixative is applied and the filter is blotted. The filter is then peeled from the slide and the slide is fixed in 95% alcohol. As a result, a monolayer of cells get deposited within an 18 mm diameter circle. Background material such as blood, inflammatory debris and mucus are eliminated. According to the manufacturer, the Cyto-Tek MonoPrep system is equivalent to automated systems and consistently produces slides with cells that exhibit optimal morphology with crisp nuclear detail and preserves the architectural features of small cell aggregates.

DIAGNOSTIC CRITERIA[1e,2]: PLEURAL AND ASCITIC FLUID

BENIGN MESOTHELIA L CELLS

Cell size	:	12-20µ diameter.
Shape	:	Round
Borders	:	Well defined or fuzzy
Nucleus	:	Central or eccentric
Nuclear shape	:	Round or reniform
Chromatin	:	Finely granular chromatin
Nucleoli	:	Single or multiple micronucleoli
Cytoplasm	:	Abundant, acidophilic/basophilic cytoplasm

Diagnostic criteria for reactive mesothelial proliferation, malignant mesothelioma and adenocarcinoma are tabulated below.(table no:8)

Table No.8: Diagnostic criteria for reactive mesothelial proliferation,malignant mesothelioma and adenocarcinoma.

Morphological Features	**Reactive Mesothelial Proliferation**	**Malignant Mesothelioma**	**Adeno carcinoma**
Arrangement	Singles/ large clusters/ Small groups	One cell/ groups	Singles & 3 dimensional Clusters
Cell cannibalism	Present	Present	Absent
Cell size &shape	Variable	Small – large round, polygonal	Medium sized
Cell borders	Well defined cell border, may appear ‘fuzzy” with small blebs.	Well defined	Well defined
Cytoplasm	Abundant, pale to dense biphasic staining with large vacuoles.	Abundant, dense, biphasic staining	Cytoplasmic vacuoles seen
6.Nucleus	Variable size Centrally/eccentrically placed nucleus. Bi/multinucleation . Sharp and delicate nuclear membrane	Central Pleomorphic	Increased nuclear cytoplasmic ratio, hyperchromasia, indendation of nucleus, irregular nuclear membrane

Morphological Features	Reactive Mesothelial Proliferation	Malignant Mesothelioma	Adeno carcinoma
7.Chromatin	Evenly dispersed	Finely granular	Uniform and granular
8.Nucleoli	Single/multiple, micro/macro nucleoli.	Single/multiple, micro/macro nucleoli.	Single/multiple Prominent
9.Others	Window formation in between the cells	Overwhelming cellularity, hemorrhagic background	Mitotic figures Seen

OVARIAN CARCINOMA

- Large cells in papillary configuration / tight clusters of cell balls with marked nuclear overlapping.
- Resembles adenocarcinoma in other respects.

GIT CARCINOMA

- Single cells/papillary clusters.
- Large cells/signet ring type cells.
- Markedly abnormal hyperchromatic eccentric nuclei.
- Prominent nucleolus.

CARCINOMA BREAST

- Large 3 dimensional clusters of round, oval morula – like cell balls with smooth outlines or papillary configuration.
- Abnormal mitosis.
- Large hyperchromatic nuclei with prominent nucleoli.
- Scanty cytoplasm.

OTHER NEOPLASMS DIAGNOSED BY CYTOLOGY OF PLEURAL AND ASCITIC FLUIDS:

- Squamous cell carcinoma
- Small cell carcinoma
- Malignant lymphoma
- Leukemia
- Malignant melanoma
- Multiple myeloma

CAUSES OF EFFUSIONS ARE DIVIDED IN TO 3 CATEGORIES

GROUP-A: DEFINITIVE DIAGNOSIS POSSIBLE UNEQUIVOCALLY BY

Cytologic Examination

1) Identification of malignant cells

2) Classification of malignant neoplasms

   a. Adenocarcinoma.

   b. Squamous cell carcinoma.

   c. Small cell carcinoma.

   d. Malignant lymphoma.

   e. Sarcoma.

   f. Large cell lymphoma.

   g. Acute leukemia.

GROUP-B: DIAGNOSTIC PITFALLS/ INCONCLUSIVE DIAGNOSIS:

- ❖ Mesothelioma versus adenocarcinoma.
- ❖ Small cell lymphoma/CLL versus reactive lymphocytosis.
- ❖ Subclassification of sarcomas.

- ❖ Subclassification of poorly differentiated neoplasms.
- ❖ Prediction of primary source of metastatic neoplasms.

GROUP-C: IMPOSSIBLE DIAGNOSIS:

- ❖ Etiology of benign effusions (with a few exceptions).

DIAGNOSTIC PITFALLS

1) Malignant Mesothelioma Versus Adenocarcinoma

Some adenocarcinomas and occasionally other neoplasms resemble malignant mesothelioma in cytologic preparations.

1) The scalloped periphery of cell clusters

2) Cell in cell arrangement

3) Intercellular windows

The above features can also be seen in other neoplasms.

So for an accurate diagnosis it is prudent to correlate the microscopic findings with the clinical and radiologic findings and to perform histochemical, immunocytochemical or electron microscopic studies.

The special stains used in most of the laboratories are: 1Mucicarmine 2. Periodic acid – Schiff (PAS) 3. Alcian blue These

stains help in determining whether the malignant cells contain neutral mucin (produced by adenocarcinomas) or hyaluronic acid (an acid mucopolysaccharide produced by mesothelial cells).Strong mucicarmine positivity indicates adenocarcinoma .Alcian blue positivity that becomes negative after treatment with hyaluronidase indicates mesothelioma. **IHC:** a strong positive staining for CEA, anti- Leu M1 and B72.3 argues against a diagnosis of mesothelioma.

2) Malignant Lymphoma Versus Benign Lymphocytosis

Recognition of a monoclonal population of lymphocytes by cell marker studies helps in malignant diagnosis of small cell lymphomas and CLL.

3) Prediction of Primary Site

This is possible in cases of small cell carcinomas, malignant melanomas and malignant lymphomas as they have distinct cytologic features. But in the majority of malignant effusions caused by adenocarcinoma, squamous cell carcinoma, poorly differentiated carcinomas, or sarcomas, the primary site of tumor cannot be determined reliably on the basis of cytological findings alone.

The accuracy of predicting the organ of origin is improved significantly, when cytologic findings are correlated with clinical data.

C- IMPOSSIBLE DIAGNOSIS BY CYTOLOGY

This group includes most benign causes, with the exception of rheumatoid arthritis, extramedullary hematopoiesis , viral, fungal and parasitic infections.

The differential diagnoses are narrowed down by determination of inflammatory cell types present in the fluid.

Purulent effusion: Pyogenic bacterial infection, acute pancreatitis or ruptured viscus.

Large number of eosinophils: post operative and traumatic conditions, repeated aspirations , spontaneous pneumothorax and peritoneal dialysate.

Predominant lymphocytes with sparsity of histiocytes and mesothelial cells: Tuberculosis.

However, these changes are not specific enough to allow an accurate diagnosis.

DAIGNOSTIC ACCURACY

Cytology is the most accurate method for diagnosis of malignant effusion.

The overall sensitivity is 70-90% according to most studies.

Closed pleural biopsy has a sensitivity of 40-70%.

But, the combination of these two methods increases the detection rate of malignancy to 80-90%.

Pleural biopsy is reserved for cases in which cytology fails to detect malignancy because of higher rate of complications.

The specificity of cytology and positive predictive value is 100% with no false positive results in most published studies.

The rate of suspicious for malignancy published varies from 2-11%.

Cytologic examination of multiple samples of an effusion increases the detection rate of cancer by 2-19% over that of one sample alone.

DIAGNOSTIC CRITERIA FOR URINE

Inflammation

- Hazy / turbid urine specimen.
- Numerous polymorphs, histiocytes, occasionally eosinophils.
- Reactive changes in the epithelial cells.
- Organisms may be present, bacterial or parasitic.
- Associated pathology, like debris in the presence of calculi.

NEOPLASMS

Benign – Urothelial Papilloma

- Increased cellularity of the urine sample.
- The cells are morphologically normal.

MALIGNANT

Grade-I Urothelial Carcinoma

- Tumors resemble normal urothelial cells.
- Increased cellularity with many single cells.
- Cells may be in loose clusters.
- Vesicular chromatin.
- Nucleoli usually absent.
- Clean background.

Grade-II Urothelial Carcinoma

- More cellularity with single cells and groups.
- Nuclear enlargement and crowding.
- Some irregularity of nuclear contours.
- Coarse chromatin.
- Clean background.

Grade III Urothelial Carcinoma

- Markedly increased cellularity.
- Malignant cells in singles, clusters and in syncytial groups.
- Cell in cell arrangement.
- Pleomorphic cells with abnormal coarse chromatin.
- Large nucleoli.
- Mitotic figures.
- Dirty background.

Other Neoplasms: Squamous cell carcinoma, adenocarcinoma, small cell carcinoma, lymphoma and malignant melanoma.

DIAGNOSTIC PITFALLS

1. Infection Versus Neoplasms

The urothelial cells in inflammatory conditions generally show reactive changes such as nuclear enlargement and some hyperchromasia but the changes are not as severe as those of malignant cells. There could be a non specific increase in the number of cells due to greater exfoliation in the presence of infection. Also, inflammatory cells can be seen abundantly in urine in high grade urothelial carcinoma. So cytology should be repeated after treating the infection.

2. False Positive Cases

- ❖ False positive cytology is often due to misinterpretation of cellular atypia which accompanies calculi, inflammation or catheterization. It is advisable to request more samples and check for the history of instrumentation / infection.

- ❖ One more cause was intravesical chemotherapy. It caused enlarged, degenerated urothelial cells with hyper chromatic nuclei which are mistaken for malignancy especially if full details are not included in the request form.

3.Iatrogenic changes

Laser treatment, radiation therapy and drugs such as cyclophosphamide, busulphan can cause changes mimicking a carcinoma.

DIAGNOSTIC ACCURACY

Accuracy depends up on

- Tumor size and number.
- Tumor grade.
- Quality of the sample.
- Preparation method
- Experience in interpretation.

The sensitivity for low-grade cancers has been demonstrated to range from 13 to 75% and over 80% for high-grade cancers. The overall sensitivity of cytology ranges from 25 to 70%. Pooled estimates from 36 studies and involving 14,260 patients demonstrated a sensitivity of 44% and a specificity of 96%[76]**.** Higher grade urothelial carcinomas are more frequently diagnosed

on cytology, while grade I tumors are more frequently missed by this method.

Between 1970 and 1983, Johnston WW et al conducted a review of cytopathologic diagnoses rendered on all malignant pleural effusions processed over a period of 14 years, and examined around 5888 fluid samples, out of which 584 were diagnosed to contain cancers cells. Among malignant pleural effusions, 75% were classified as carcinomas. Large cell undifferentiated carcinoma and lymphoma/leukemia approximated 14.3% and 15% respectively. Adenocarcinomas comprised 47% of the 584 specimens[77].

Cytology examination of not only body fluids but urine, also yields important diagnostic information. Koss and associates in a study of cytological examination of voided urine in 183 patients with suspected tumors, reported an overall sensitivity rate of 82% [78].

In 1993 Venrick MG and Sidawy MK performed a double-blind retrospective review of 90 pleural and ascitic fluids to determine the optimal number of smears necessary to produce an accurate evaluation, and to analyze the utility of different preparation techniques like four Papaniculoau smears and one

Diff – Quick stained smears for 40 positive and 50 negative cases. Their showed that the diagnostic yield of 17 evaluating 5smears compared with 3(one of each preparation) is identical and air-dried smears were the most sensitive in identifying malignant cells and infectious organisms [79].

Pleural fluid cytology and pleural biopsy results were studied by Kumar ND et al in 65 cases of pleural effusion in 1995. The efficacy of pleural biopsy in diagnosis of neoplastic and non-neoplastic pleural diseases was compared. Of the 24 cases with confirmatory evidence of cancer, 17(70.8%) had positive cytologic findings in pleural fluid, whereas pleural biopsy was diagnostic in only 13 cases (54.1%). For non-malignant pleural effusion out of 41 cases, 40 (97.5%) had a definite diagnosis (tuberculous pleuritis, acute fibrinous pleuritis or hydatid cyst) which could be made by cytology while only 31 (75.6%) out of 41 were diagnosed on pleural biopsy. They concluded that cytologic evaluation of pleural fluid is more efficacious in the diagnosis of malignant and non-malignant pleural disease than percutaneous pleural biopsy[80].

Ong et al conducted a cytological examination of pleural fluids on 103 patients with suspected malignant pleural effusions in 2000. A first sample cytology in their study was positive in 48.5%

of patients. The percentage positivity increased with repeated pleural fluid cytology examination on same set of patients. It was concluded that pleural fluid cytological examination is a useful initial step in the diagnostic work up of patients with malignant pleural effusions [81].

In 2001 Bjelakovic et al in a study on 90 patients with ascites, in order to determine the value of a cytological analysis in the differential diagnoses of ascites, found malignancy to be the cause of ascites in 33.33% of patients [82].

In a review study by Yamamoto et al in 2003 on 189 patients who underwent curative resection for carcinoma of the colon where a peritoneal cytology was performed before manipulation of the tumor intra-operatively, the authors concluded that conventional peritoneal cytology not only helps in the initial diagnosis but also serves as a new prognostic marker after curative resection and is useful in predicting peritoneal recurrence[83].

Jha R, Shrestha H, Sayami et al conducted a study in 2006 to evaluate the sensitivity of ascitic fluid in detecting malignancy. Out of 65 cases, total 21 effusions (32.3%) were tumor cell positive.

Ascitic fluid cytology had sensitivity of 56.7% and specificity of 100%[84].

Pedamallu S and Alexandrou K in 2010 conducted a retrospective study to compare urinary cytology with pathologic findings of proven bladder cancer patients to determine the value of voiding urinary cytology. They did a study of voided urinary cytology results and histopathology reports in 169 proven bladder cancer patients.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology at Stanley Medical College from June 2012 to June 2014 .Approval of the Ethical committee of Stanley Medical College & Hospital was obtained.

In our study we proposed to conduct a comparative analysis of cytology of body cavity fluids by using conventional methods and Liquid Based Cytology methods. Fluid samples collected in the clinical wards and submitted to our laboratory were studied. Totally 100 fluid samples were randomly taken and studied. Smears were prepared by both conventional method and Liquid Based cytology methods from all the 100 samples. All these smears were screened and a comparative analysis was made between the conventional and liquid based methods.

COLLECTION OF SAMPLES

Three different body cavity fluids were used in the study. They include pleural fluid, peritoneal fluid and urine. Each fluid is obtained by different techniques performed in the clinical wards.

Pleural fluid was obtained by thoracocentesis. Thoracocentesis was done by inserting a needle in the sixth or seventh intercostal space .

The peritoneal fluid was obtained by inserting a needle into the abdominal wall at the most dependent portion of the fluid accumulation.

Random voided urine samples were used. The samples were sent with a label and an appropriate requisition. All the fluid samples were divided into 2 parts, one part was subjected to conventional method and the other part was subjected to Liquid based cytology.

CONVENTIONAL METHOD

This technique is routinely done in our laboratory. The samples were received in a test tube. The volume, colour and the transparency of the fluids were recorded. The fluid samples were

centrifuged at 2000 rpm for 10minutes.The supernatant was discarded. The sediment was directly applied on the glass slide and a smear was made with another slide. The smear was fixed in isopropyl alcohol for 15minutes and stained with hematoxylin and eosin.

Staining Procedure

1) Hematoxylin- 5 – 7 minutes
2) Tap water wash
3) 1%Acid alcohol – 1 dip
4) Blueing
5) Eosin-15 -30secs
6) Blot dried and mounted with DPX.

LIQUID BASED CYTOLOGY

Step1: Sample centrifuged at 2200-3000 rpm for 10mts
Supernatant and pellet was formed

↓

Step2 : Discard the supernatant
Vortex the pellet

↓

To the pellet 0.5ml of lyser solution added

↓

Step3: Recentrifuge (2200-3000rpm for 5 mts)
Pellet and supernatant formed

↓

Step4: Discard the supernatant
Pellet was vortexed

↓

Step5: on a normal slide , 1 drop of cell base and
30- 40 microlitre of the vortexed sample were added together

↓

A circular smear of 20mm diameter made Air dry the smear
Stained with hematoxylin and eosin

Note: Lyser solution removes mucus and blood.

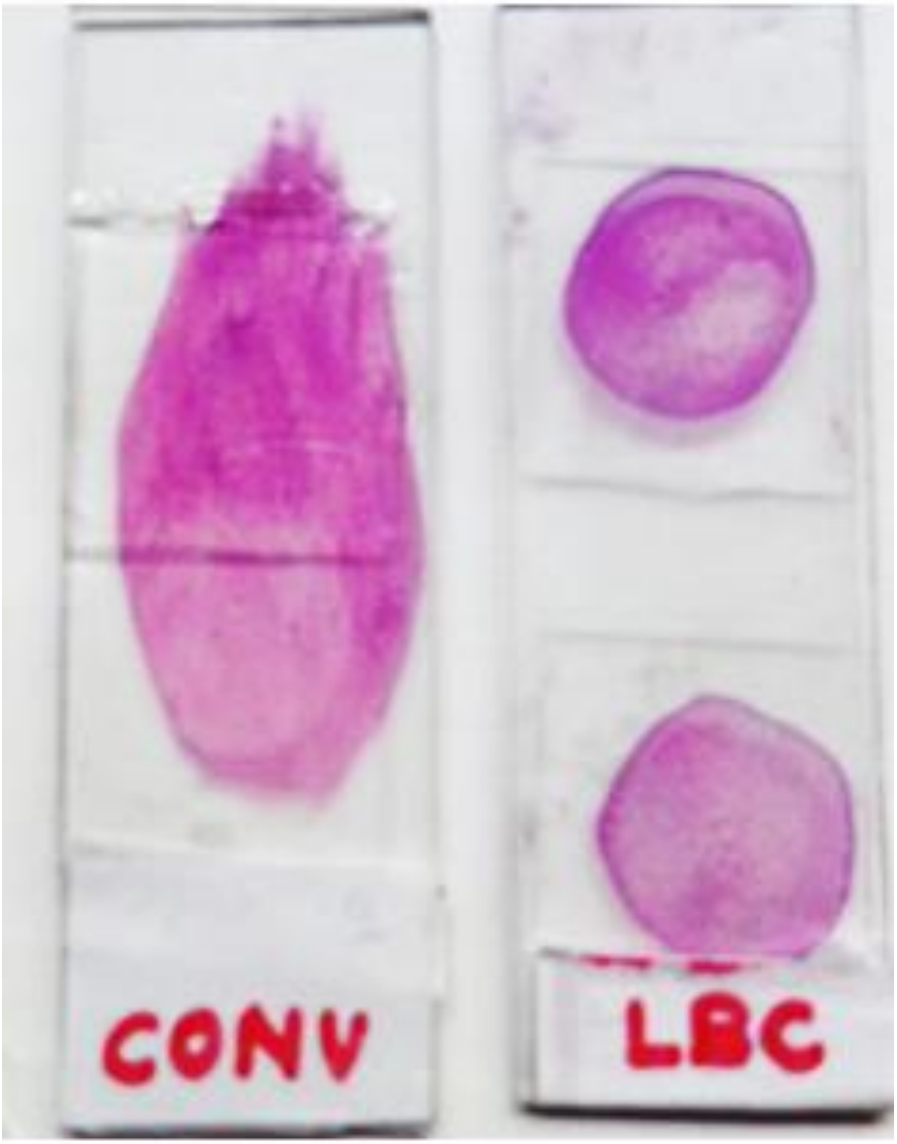


Fig-3: slides prepared by conventional and liquid based cytology techniques

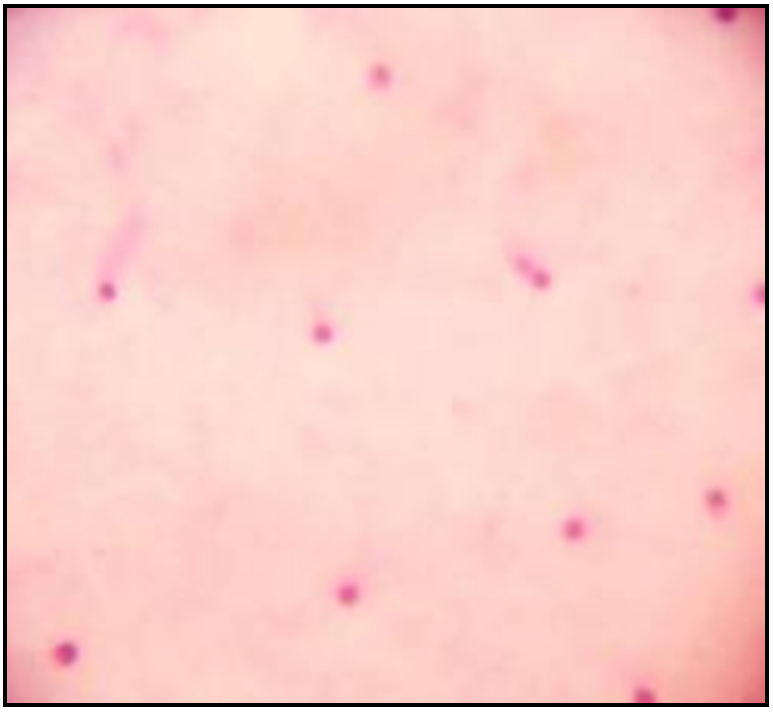


Fig 4: Less Cell yield in conventional smears

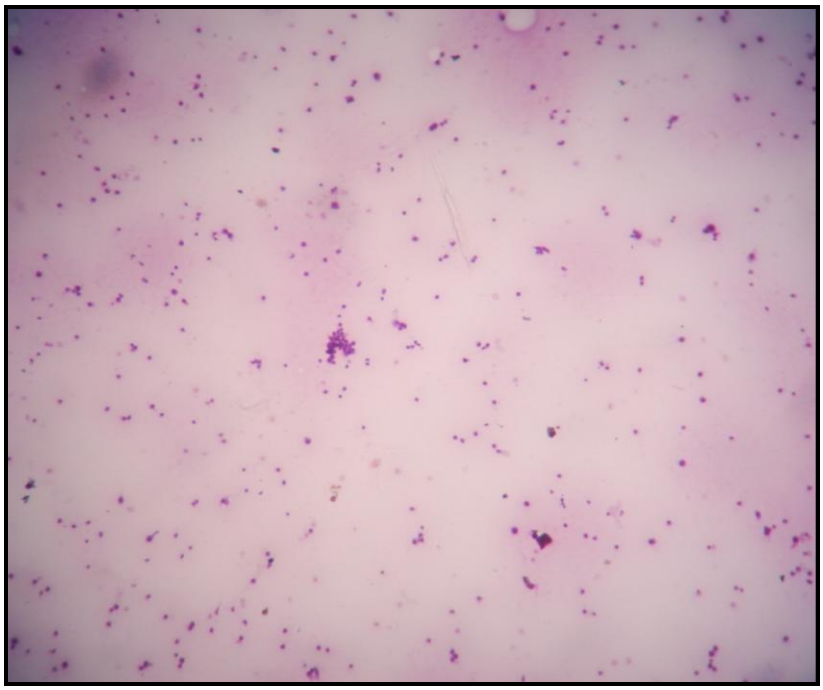


Fig 5: Increased cell yield in liquid based cytology smears

Fig 6: Sparse cellularity in conventional smears of urine

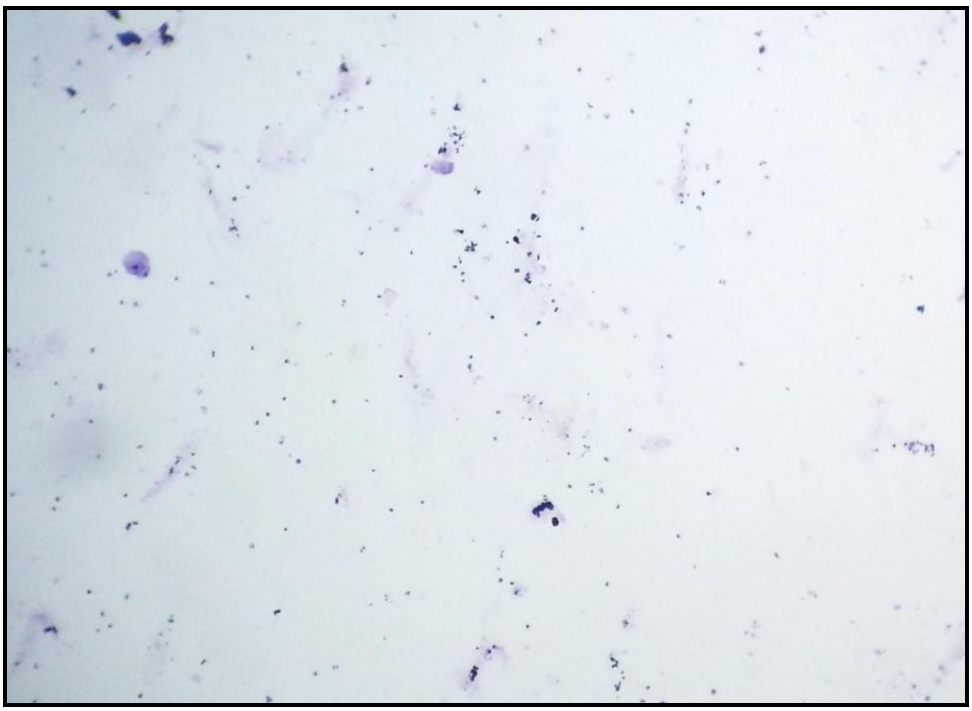


Fig 7: Increased cellularity in Liquid based cytology smears of urine

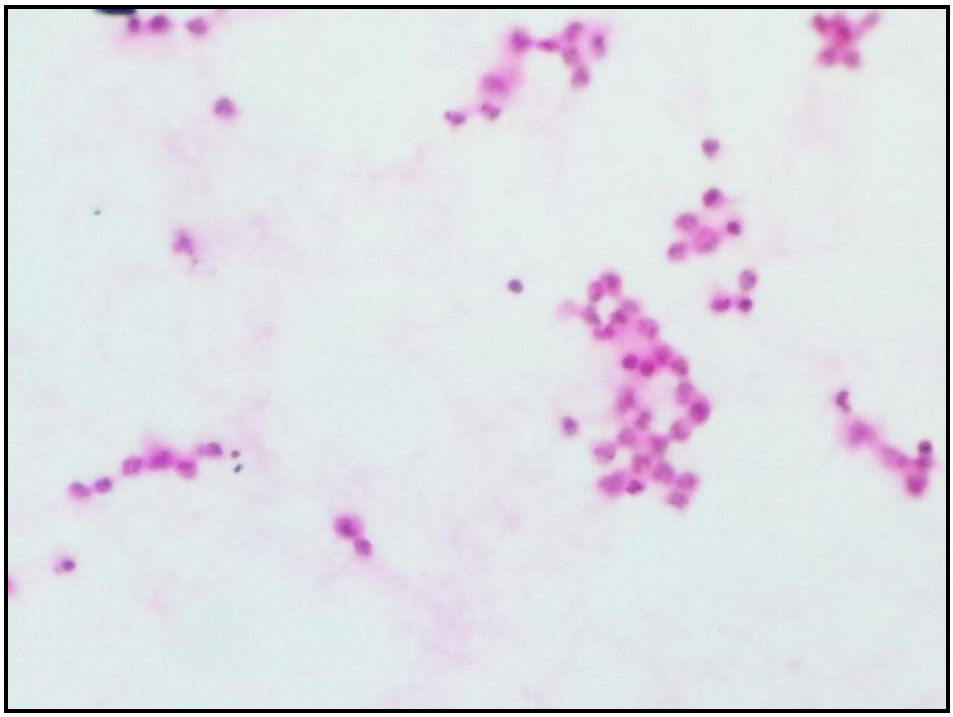


Fig 8: Poor cell preservation in conventional smears

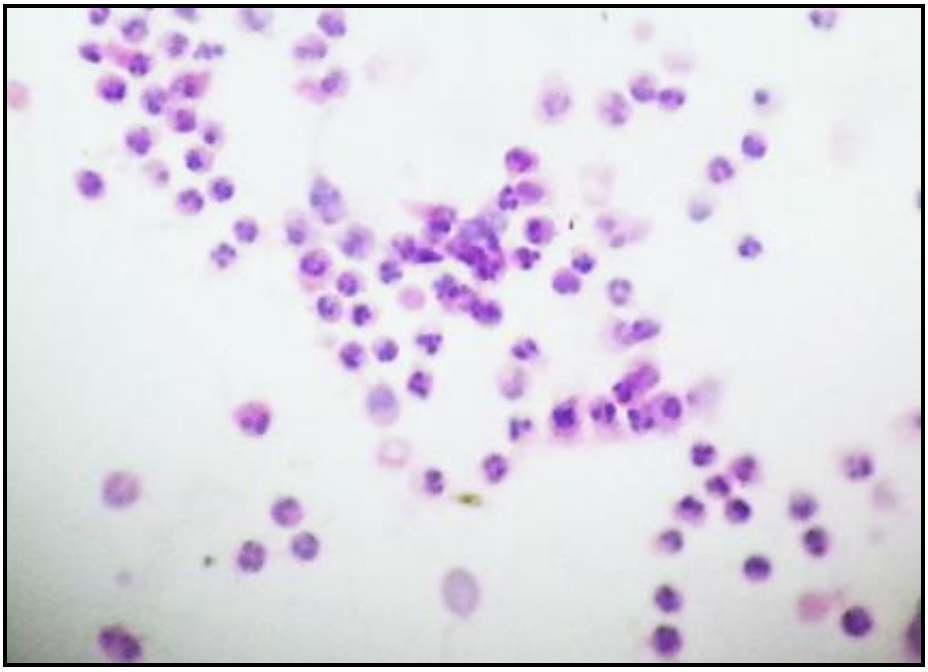


Fig 9.Excellent preservation of cell morphology in liquid based smears

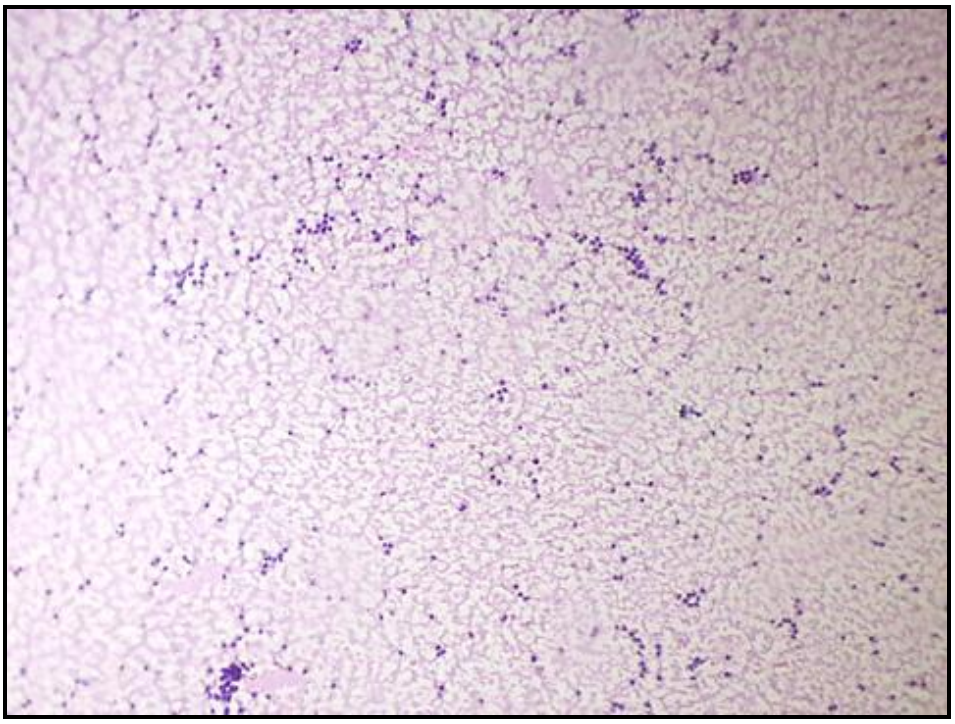


Fig 10.Background proteinaceous material in liquid based cytology smears

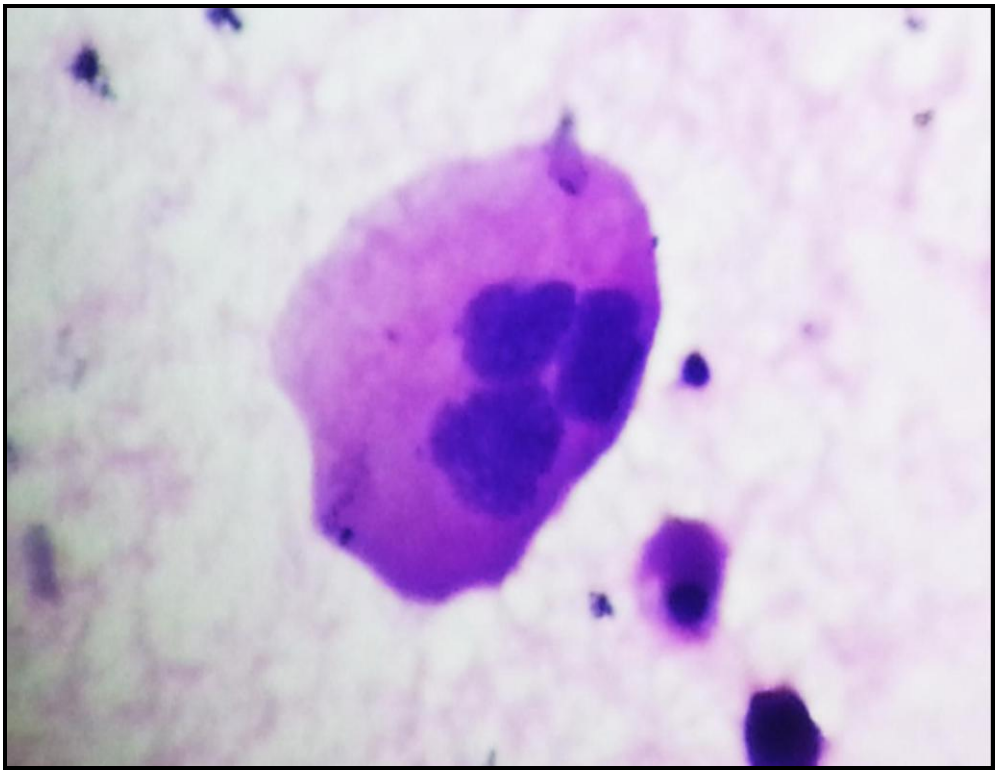


Fig 11.Malignant cell with irregular nuclear membrane and abnormal chromatin distribution in Liquid based cytology smears

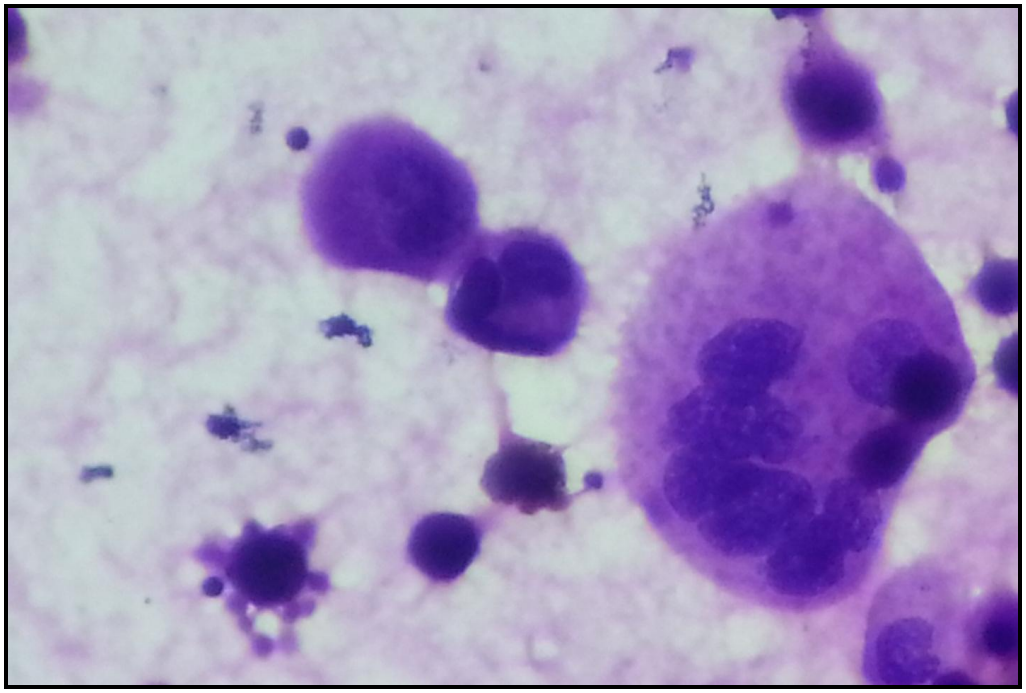


Fig 12.Tumour giant cell seen in Liquid based cytology smears

OBSERVATION & RESULTS

Total No. of samples subjected to cytological examination: 100

DISTRIBUTION OF CASES

33 were pleural fluids, 56 were peritoneal fluids and 11 were urine samples (see table 9 and fig. 13)

Table No.9: Table showing distribution of cases

Type of fluid	Pleural	Peritoneal	Urine
No:of Cases	33	56	11

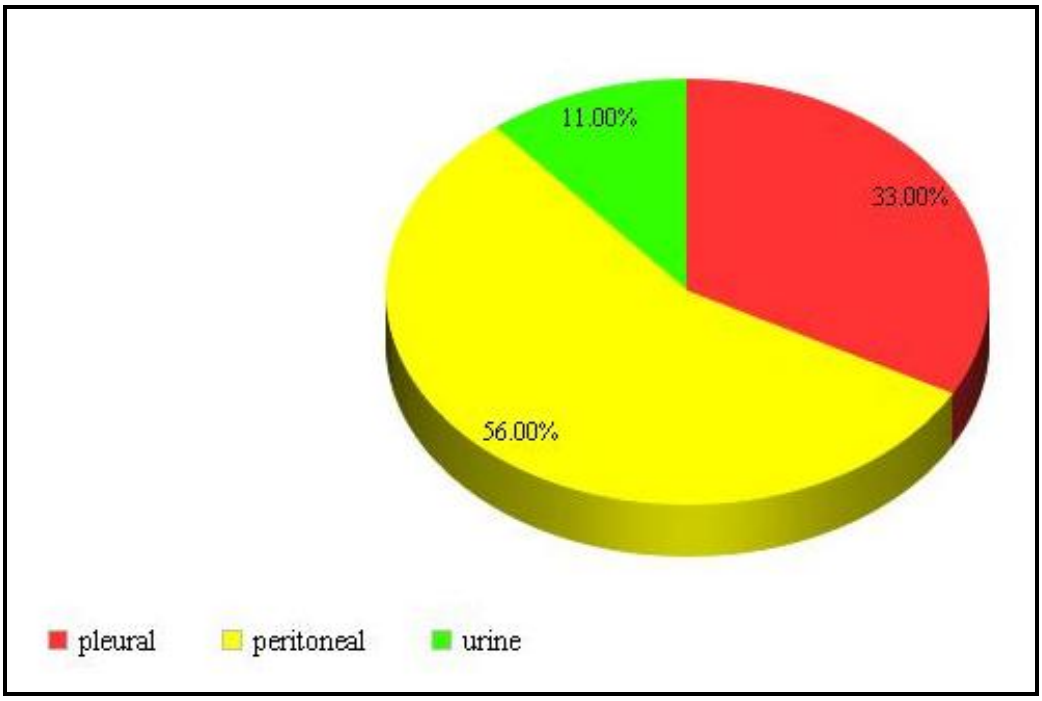


Fig 13: Pie chart showing distribution of cases.

SEX DISTRIBUTION

Out of the total 100 samples, 77were from males and 23 were from females. (See fig. 14)

The distribution of samples among males was as follows: 29 pleural fluids, 38 peritoneal fluids and 10 urine samples.

The distribution of samples among females was as follows: **4** pleural fluids, 18 peritoneal fluids and 1 urine sample.

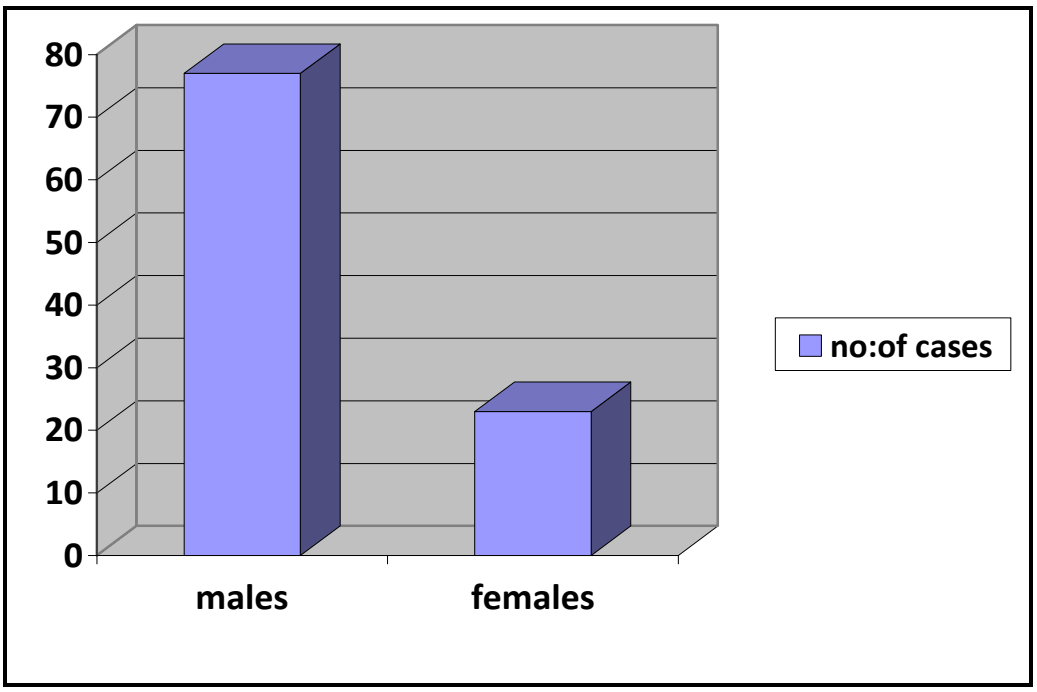


Fig14: Bar diagram showing sex distribution of cases

AGE DISTRIBUTION

Age distribution of the cases are tabulated as follows(table no:10 & Fig 15)

Table.No.10: Table showing age distribution

Age Category	No:of Cases Observed
0-20 Yrs	4
20-40 Yrs	25
>40 Yrs	71

Among 100 cases, 4 cases belonged to 0-20 years, 25 cases belonged to 20-40 years and 71 cases belong more than 40 years.

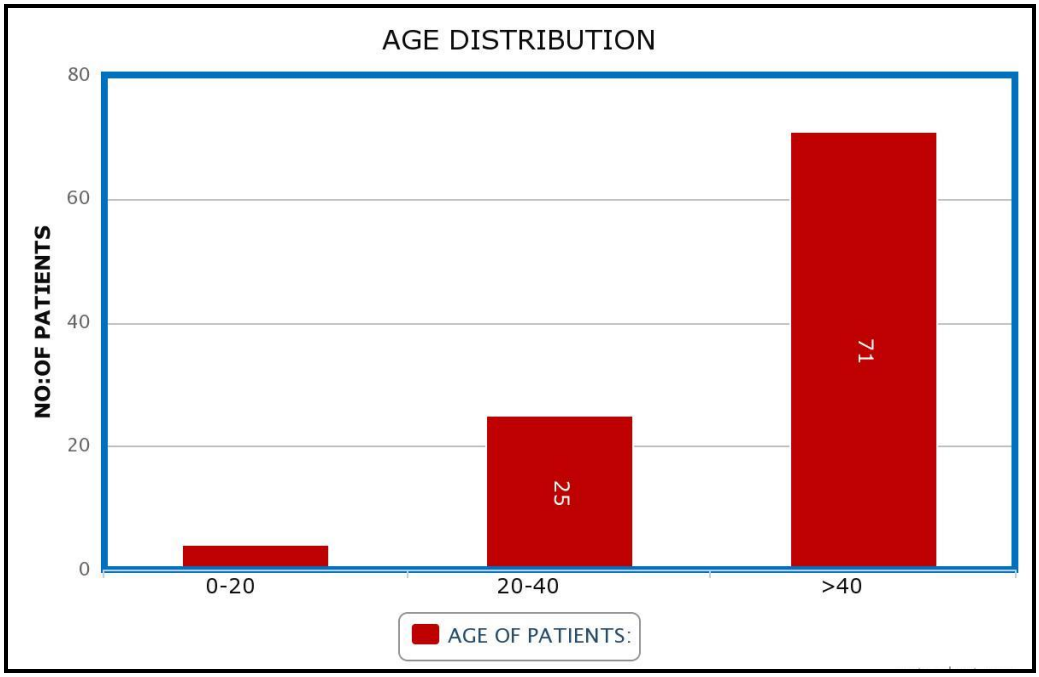


Fig 15: Bar diagram showing age distribution of cases

DISTRIBUTION OF CASES WITH REGARD TO MALIGNANT AND NON-MALIGNANT SAMPLES

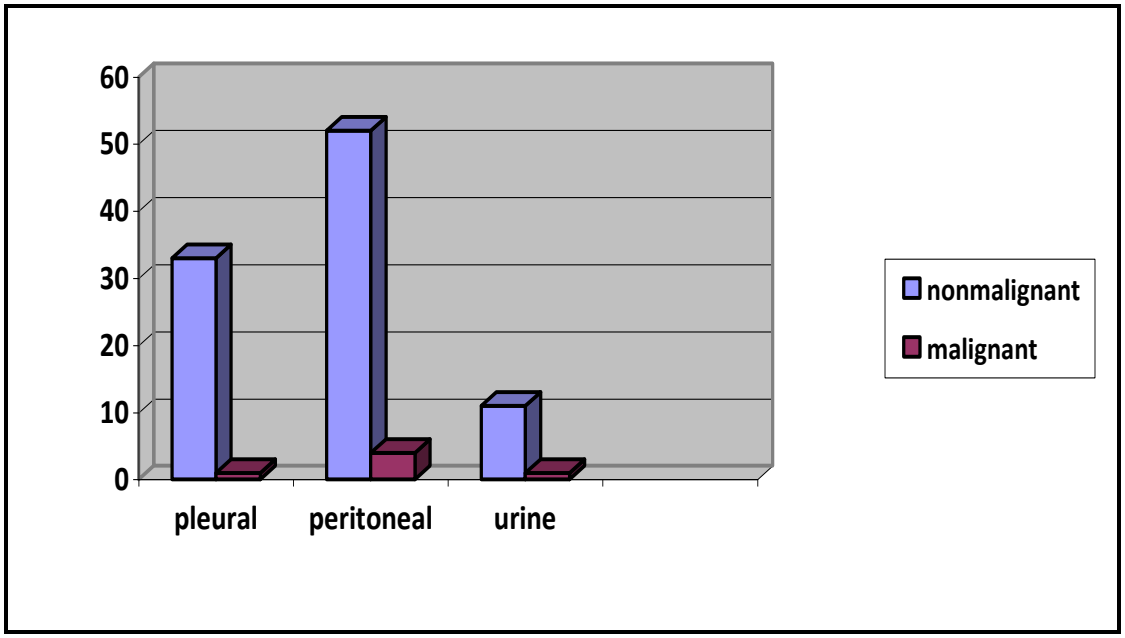


Fig 16: Bar diagram representing distribution of non-neoplastic and neoplastic samples

In each category – (Refer fig. 16)

1) ***Pleural fluid:*** Out of the 33 cases, 1 was malignant.

2) ***Ascitic Fluid:*** Out of 56, 4 were malignant.

3) ***Urine:*** 1 was malignant out of 11 cases.

EVALUATION OF MORPHOLOGICAL PARAMETERS

Both the conventional centrifuged smears and LBC smears were examined. (Fig No:3) The morphological features like cell yield, cell morphology, cell distribution and background were analysed and scored, as 0 to 2+ as per Archana et al.[85] (See Table No:11)

Table No.11: Scoring System

Parameter	Quantitative Description	Point Score
Background blood or proteinaceous material	1.Large amount, great compromise in diagnosis	0
	2.Moderate amount, diagnosis possible.	1
	3.Minimal, diagnosis easy.	2
Amount of cellular material	1.Minimal to absent, diagnosis not possible.	0
	2.Sufficient for cytodiagnosis.	1
	3.Abundant, diagnosis simple.	2
Cell morphology, cellular degeneration and trauma	1.Marked cellular degeneration, diagnosis not possible.	0
	2.Moderate cellular degeneration, diagnosis possible.	1
	3.Minimal cellular degeneration, diagnosis easy.	2
Distribution of cells	1.Totally in the periphery or sparsely distributed.	0
	2.Combination.	1
	3.Evenly distributed.	2

Statistical analysis: Wilcoxon rank sum test was used to determine the statistical significance of difference of each parameter between two methods. The values analysed by this test provides results in the form of positive ranks, negative ranks and tie. Positive ranks mean LBC is superior to CS. Negative ranks mean CS is superior to LBC. Tie means both are same.

PLEURAL FLUID: TOTAL NO OF CASES-33

Table no.12: Comparison of cell yield:

Method	Score 0	Score 1	Score 2
Conventional	2 (6%)	24(73%)	7 (21%)
LBC	2 (6%)	14 (42%)	17(52%)

CS showed a cellularity score of 0 in 2 cases, 1 in 24 cases and 2 in 7 cases. LBC showed a cellularity score of 0 in 2 cases, 1 in 14 cases, 2in 17 cases The maximum cellularity score 2 was seen in the LBC smears of 17 cases whereas it was observed in 7 cases of CS.

Table No: 13 Comparison of Cell Morphology

Method	Score 0	Score 1	Score 2
Conventional	1(3%)	21 (64%)	11(33%)
LBC	1 (3%)	6 (18%)	26 (79%)

Cell morphology in CS showed score 0 in 1 case, score 1 in 21 cases and score 2 in 11 cases. Cell morphology in the LBC showed score 0 in 1 case, score 1 in 6 cases and score 2 in 26 cases.(Table No:13)

Table No 14: Comparison of Cell Distribution

Method	Score 0	Score 1	Score 2
Conventional	6(18%)	22 (67%)	5(15%)
LBC	4 (12%)	6 (18%)	23 (70%)

Cell distribution in CS showed score 0 in 6 cases, score 1 in 22 cases and score2 in 5 cases. Cell distribution in LBC showed score 0 in 4 cases, score 1 in 6 cases and score2 in 23 cases.

Table no 15: Comparison of Background

Method	Score 0	Score 1	Score 2
Conventional	5 (15%)	19 (58%)	9 (27%)
LBC	3 (9%)	19 (58%)	11 (33%)

With regard to background, CS showed score 0 in 5 cases, score 1 in 19 cases and score 2 in 9 cases and LBC showed score 0 in 3 cases, score 1 in 19 cases and score 2 in 11 cases.

STATISTICAL ANALYSIS OF ALL THE MORPHOLOGICAL PARAMETERS OF PLEURAL FLUID:

According to Wilcoxon rank sum test, the results are analysed and tabulated as follows.

Table No.16: Statistical Analysis of all the Morphological Parameters of Pleural Fluid

Parameters	Superior LBC	Superior Conventional	Equivalent	p value
Cellularity	11 (33%)	1(3%)	21(64%)	0.004
Cell morphology	15 (45%)	0	18 (55%)	0.000
Cell distribution	21 (64%)	1(3%)	11(33%)	0.000
Background	7 (21%)	3(9%)	23(70%)	0.206

INFERENCE

- Cell yield is better in LBC than conventional. The results showed that there is statistically significant difference between the two methods. ($p<0.05$)(see fig.4&5)

- Cell morphology is better preserved in smears obtained by LBC than conventional. These results show statistically significant difference between the methods.($p<0.05$)

- Liquid based cytology demonstrated more uniform distribution of cells than conventional, which was statistically significant.($p<0.05$)

- Background: These results are not statistically significant ($p>0.05$). Hence background was not comparable between the two methods.(refer table no:16)

PERITONEAL FLUID: TOTAL NO OF CASES-56

Table No.17:Comparison of cell yield

Method	Score 0	Score 1	Score 2
Conventional	5 (9%)	40 (71%)	11(20%)
LBC	4 (7%)	28 (50%)	24 (43%)

CS showed a cellularity score of 0 in 5 cases, 1 in 40 cases and 2 in 11 cases. LBC showed a cellularity score of 0 in 4 cases, 1 in 28 cases, 2 in 24 cases .The maximum cellularity score 2, was seen in the LBC smears of 24 cases where as, it was observed in 11 cases of conventional smears.

Table No.18: Comparison of Cell Morphology

Method	Score 0	Score 1	Score 2
Conventional	5 (9%)	38 (68%)	13 (23%)
LBC	3 (5%)	13 (23%)	40 (72%)

Cell morphology in CS showed score 0 in 5 cases, score 1 in 38 cases and score 2 in 13 cases. Cell morphology in the LBC method showed score 0 in 3 cases, score 1 in 13 cases and score 2 in 40 cases.

Table No: 19 Comparison of Cell Distribution

Method	Score 0	Score 1	Score 2
Conventional	9 (16%)	35 (63%)	12 (21%)
LBC	4 (7%)	21 (38%)	31 (55%)

Cell distribution in CS showed score 0 in 9 cases, score 1 in 35 cases and score 2 in 12 cases. Cell distribution in LBC showed score 0 in 4 cases, score 1 in 21cases and score 2 in 31 cases.

Table No.20:Comparison of Background

Method	Score 0	Score 1	Score 2
Conventional	6 (11%)	42 (75%)	8 (14%)
LBC	5 (9%)	40 (71%)	11 (20%)

Background in CS showed score 0 in 6 cases, score 1 in 5 cases and score 2 in 9 cases. Background in LBC method showed score 0 in 5 cases, score 1 in 40cases and score 2 in 11 cases.

STATISTICAL ANALYSIS OF ALL THE MORPHOLOGICAL PARAMETERS OF PERITONEAL FLUID:

According to Wilcoxon rank sum test, the results are analysed and tabulated as follows.

Table No.21: Statistical analysis of all the morphological parameters of peritoneal fluid

Parameters	Superior LBC	Superior Conventional	Equivalent	p value
1.Cellularity	19(34%)	6 (11%)	31(55%)	0.009
2.Cell morphology	34 (61%)	5 (9%)	17(30%)	0.001
3.Cell distribution	29(52%)	6 (10%)	21(38%)	0.000
4.Background	9(16%)	5 (9%)	42 (75%)	0.285

INFERENCE

- Cell yield is better in LBC than conventional. The results showed that there is statistically significant difference between the two methods. ($p<0.05$)
- Cell morphology is better preserved in smears obtained by LBC, than conventional. These results show statistically significant difference between the methods.($p<0.05$)(see fig 8 &9)
- Liquid based cytology demonstrated more uniform distribution of cells than conventional, which was statistically significant.($p<0.05$)
- Background:These results are not statistically significant.($p>0.05$)Hence background was not comparable between the two methods.(see fig 10)

URINE: TOTAL NO OF CASES-11

Table No.22: Comparison of cell yield

Method	Score 0	Score 1	Score 2
Conventional	5 (45%)	4 (37%)	2(18%)
LBC	0	8 (73%)	3 (27%)

CS smears showed a cellularity score of 0 in 5cases, 1 in 4 cases and 2 in 2 cases. LBC showed a cellularity score of 0 in none of cases, 1 in 8 cases, 2in 3 cases (table no:22)

Table No: 23 Comparison of Cell Morphology

Method	Score 0	Score 1	Score 2
Conventional	3 (27%)	7 (64%)	1 (9%)
LBC	0	7 (64%)	4 (36%)

Cell morphology in CS showed score 0 in 3 cases, score 1 in 7 cases and score2 in 1 cases. Cell morphology in the LBC method showed score 0 in none of the cases, score 1 in 7 cases and score2 in 4cases.(table no:23)

Table No.24: Comparison of Cell Distribution:

Method	Score 0	Score 1	Score 2
Conventional	5 (45%)	5 (45%)	1 (10%)
LBC	1 (10%)	6 (55%)	4 (35%)

Cell distribution in the conventional centrifugation method showed score 0 in 5 cases, score 1 in 5 cases and score2 in 1case.Cell distribution in the LBC method showed, score 0 in 1 case, score 1 in 6cases and score2 in 4 cases.(table no:24)

TableNo .25: Comparison of Background:

Method	0	1	2
Conventional	2 (18%)	7 (64%)	2 (18%)
LBC	0	8 (73%)	3 (27%)

Regarding background, CS showed score 0 in 2 cases, score 1 in 7 cases and score2 in 2 cases. Background in LBC method showed score 0 in none of the cases, score 1 in 8 cases and score2 in 3 cases.

STATISTICAL ANALYSIS OF ALL THE MORPHOLOGICAL PARAMETERS OF URINE:

According to Wilcoxon rank sum test, the results are analysed and tabulated as follows.

Table No.26: Statistical analysis of all the morphological parameters of urine

Parameters	Superior LBC	Superior Conventional	Equivalent	p value
Cellularity	6 (55%)	0	5 (45%)	0.014
Cell morphology	6 (55%)	0	5 (45%)	0.014
Cell distribution	6 (55%)	0	5 (45%)	0.020
Background	4(36%)	1(9%)	6 (55%)	0.180

INFERENCE

- Cell yield is better in LBC than conventional. The results showed that there is statistically significant difference between the two methods. ($p<0.05$)(see fig 6&7)
- Cell morphology is better preserved in smears obtained by LBC than conventional. These results show statistically significant difference between the methods.($p<0.05$)
- Liquid based cytology demonstrated more uniform distribution of cells than conventional, which was statistically significant.($p<0.05$)
- Background: These results are not statistically significant.($p>0.05$)Hence background was not comparable between the two methods.

OTHERS

Among 100 samples, only 6 were malignant effusions. LBC gave a definitive diagnosis in all the 6 cases whereas CS diagnosed only 2 cases. Out of the 6 malignant effusions, 4 were peritoneal, 1 pleural and 1 urine.

Out of the 6 malignant effusions, 4 were obtained from males and 2 from the females. The age of the patients ranged from 45-65 years.

On follow-up , in 3 out of 6 cases , the primary was proven by biopsy. The primaries were periampullary carcinoma, sigmoid colon carcinoma and carcinoma stomach.

In all the 6 cases, cellularity, cell morphology and uniform cell distribution was better in LBC than CS, hence more number of cases were diagnosed by LBC. (see fig 11 &12)

DISCUSSION

The body cavities in human are lined by the two layers of mesothelium- visceral and parietal. There are three important cavities which includes - the pleural covering the lungs, the peritoneal enclosing gastrointestinal tract organs and pericardial covering the heart. In the absence of disease the two layers of these cavities are separated by a thin layer of lubricating fluid to facilitate the movements of the membranes against one another [1d]

In disease conditions, excess fluid accumulates within these cavities constituting effusion which may either be a transudate or exudate.

Investigations of the effusions by cytologic examination are of much importance in the diagnosis of diseases as well as for exclusion of neoplasia. A cytologic examination of the fluid performed on the smears of centrifuged specimen helps in differentiating benign from malignant effusions. It also aids in establishing the nature of malignancy in many cases and helps in the planning of treatment .It eliminates the need for invasive procedures and unnecessary surgical intervention, thus making the

pathologist contribute positively to the clinical diagnosis and management of patients.

There are many techniques used in the processing of the fluid specimens. They include the conventional centrifugation, cytospin preparations, membrane filtration and cell blocks. Newer techniques have evolved based on the concept of thin-layer cytology. These techniques are known as Liquid based cytology. The type of technique to be adapted by a laboratory depends upon its needs. Majority of the laboratories still use the conventional techniques.

Liquid based cytology improves the quality of smears by means of an improved way of slide preparation following collection of samples in a standard way. It provides more representative sample of specimen with reduced obscuring background material which allows faster and more reliable screening.

In recent years liquid-based is becoming an alternative to conventional cytopreparatory methods. These methods are preferred over conventional methods in view of its benefits like better cellularity, clean background, diagnostic accuracy,

cytopreservability, uniform distribution of cells and decreased screening time.

In our laboratory, we routinely use conventional centrifugation technique for preparing smears from fluid samples. But we are posed with much difficulties in interpreting these fluids because of the decreased cellularity and poor preservation of morphology of the cells. Hence in our laboratory we conducted a study comparing the conventional technique with the Liquid Based Cytology technique. The smears were compared on the morphological parameters such as cellularity, cytomorphology, cell distribution and background.

CELL YIELD

The number and type of cells give information about the target tissue.The cell yield is influenced by lesional and lesional factors particularly type of sampling method employed.

In our study , LBC showed better cellularity than CS in all the 3 types of fluids such as pleural, peritoneal and urine. Better cellularity helps in early diagnosis and it also eliminates the need for repeat tap.

In a study conducted by Seung et al[75] using 713 urine samples, LBC showed better cellularity than CS in 63.2% of cases whereas same cellularity in 36.8%. This correlated with our study where LBC showed better cellularity than CS in 55% of cases and same cellularity in 45% of urine samples.(Refer Table No:26)

Another study conducted by Babloyan et al[63] using 110 peritoneal fluids demonstrated that LBC (52%) showed better cellularity as compared to CS (9%) and same cellularity in 39% of cases. Accordingly, in our study also LBC (34%) showed better cellularity than CS.(11%) among the peritoneal fluid samples.(Refer Table No:21)

Another study conducted by Alwahaibi et al[62] using 17 pleural and 24 peritoneal fluids demonstrated that CS were cellular in 78% of cases and LBC showed high cellularity in only 2% of cases. In contrast to this study, CS were cellular in 20% of cases and LBC showed high cellularity in 46% (score 2) (refer table no:12 & 17) among 33pleural and 56 peritoneal fluids used in our study.

CELL MORPHOLOGY

In our study, one important finding was good preservation of cytomorphologic details in LBC as compared to CS in all 3 types of fluids.

Cytomorphological preservation plays an important role for the identification of the diagnostic cells. It is used to differentiate benign from malignant cells(Bong et al 2008)[61].

A study conducted on urine samples by Seung et al[75], demonstrated that cytomorphologic features are better preserved in LBC than CS in 47.4% of cases and same by both methods in 52.6% of cases in urine samples. In our study, cytomorphology was better preserved in LBC than CS in 55% of cases and same by both methods in 45% of cases (refer table no:26) which is in accordance with the former study.

Another study conducted by Babloyan et al[63] using 110 peritoneal fluids demonstrated that LBC showed excellent cytomorphology in 46% of cases whereas CS in 6% of cases. In agreement to the above study, our study also demonstrated LBC (72%) superior to CS (23%) in terms of excellent cytomorphology.(score 2) (Refer table no:18)

CELL DISTRIBUTION

Uniform cell distribution provides a better visual image of the disease process. It will help in easy and rapid screening and decreases the number of unsatisfactory specimens.

In our study, Liquid based cytology demonstrated more uniform distribution of cells than conventional, which was statistically significant.($p<0.05$)

Bong et al[61] conducted a study of 120 urine samples and found that CS was superior to LBC in terms of cell distribution. ($p<0.05$). In contrast, our study showed more uniform cell distribution in LBC than CS in the urine samples. ($p<0.05$) (Refer table no:26)

A study conducted by Seung et al[75] using urine samples,showed LBC demonstrated more uniform distribution than CS in 73.7% of cases. Similarly, our study also LBC showed uniform distribution than CS in 55% of cases.(refer table no:26)

Another study conducted by Alwahaibi et al[62] using pleural and peritoneal fluids, LBC showed uniform cell distribution in 98% of cases and CS showed only in 27% of cases .Accordingly, in our study LBC also showed uniform cell distribution in 61%% of

cases and CS in only 9% of cases. (score 2 of pleural and score 2 of peritoneal fluids)(refer table no: 14& 19)

BACKGROUND

In our study, even though clean background were seen in more number number of cases in LBC than CS in all 3 types of fluids, they were not comparable because the results were not statistically significant.

According to study conducted by Babloyan et al[63] with peritoneal fluids, LBC showed obscuring background in 12% of cases and CS in 69% of cases and they concluded that LBC produced more cleaner background compared to CS.In our study , LBC showed obscuring background (score 0) in 9% of cases and CS in 11% of cases(Refer table no:20).But the results were not statistically significant.($p>0.05$)

In the study done by Seung et al[75] using urine samples , CS showed obscuring background in 3.8% of cases and LBC in none of the cases.In our study, (score 0) CS (18%)showed more cases with obscuring background than LBC(refer table no:25). The results were not statistically significant.

A study conducted by Alwahaibi et al[62] using pleural and peritoneal fluids observed that LBC produced clean background in 78% of cases and CS in 17% of cases.(refer table no:15 & 20) But our study was inconclusive because of the lack of clinical significance.

OTHERS

In our study, out of the 33 pleural fluid samples, one case was diagnosed as malignancy by both LBC and CS. Among the 56 peritoneal fluids, 4 were diagnosed as malignancy by LBC and 1 by CS. Our study included 11 samples of urine and malignant cells were seen in only 1 sample by both LBC and CS.

SUMMARY

- This study was conducted to compare the performance of LBC and CS in body cavity fluids like pleural ,peritoneal fluids and urine.
- Totally 100 fluid samples were subjected to cytologic examination, out of which 33 were pleural fluids, 56 peritoneal fluids and 11 urine samples
- Majority of the samples were non-neoplastic (94 out of 100).
- Among 94 non-neoplastic samples, 32 (34%) were pleural fluid,52 (55%) were peritoneal and 11 were urine.(11%)
- Out of the 100 fluid samples, 6 were malignant effusions.
- Among 6 neoplastic effusions, 1 (17%)was pleural, 1 (17%) was urine and 4 were peritoneal.(67%)
- Four morphological parameters were compared between CS and LBC like cell yield, cell morphology, cell distribution and clean background.

- Cell Yield: LBC showed better cell yield as compared to CS in pleural, peritoneal fluids and urine. The results were statistically significant .($p<0.05$)

- Cell Morphology: Cell morphology was better preserved by LBC than CS in pleural, peritoneal fluids and urine. This was proved significant by p value less than 0.05.

- Cell Distribution: LBC showed more uniform cell distribution as compared to CS in pleural ,peritoneal fluids and urine. These results showed a statistically significant difference between the two methods.($p<0.05$)

- Background: The results obtained from both methods were not statistically significant. ($p>0.05$).Hence LBC was not comparable to CS in terms of background. This was true for pleural, peritoneal fluids and urine.

CONCLUSION

Liquid based cytology was found superior to conventional smears in terms of cell yield, preservation of cell morphology and uniformity of cell distribution. Liquid based cytology helped in diagnosing more number of malignant effusions than conventional smears. Hence, Liquid based cytology can be preferred to conventional smears for cytologic examination of body cavity fluids. With regard to typing the characteristics of malignant effusions, more samples have to be analysed and a separate study is required.

REFERENCES

1) Koss LG and Melamed MR (Eds). Koss' Diagnostic cytology and its histopathologic bases.5th edition, Philadelphia: JB Lippincott Company;2006.pp11(a); 12(b); 950(c);1575 (d) 922-1016 (e)

2) Ehya H.Effusion cytology. Clinics in laboratory medicine. 1991; 11(2): 443-67.

3) Kavatkar AN,Nagwanshi CA and Dabak SM. "Study of a Manual Method of Liquid Based Cervical Cytology" Indian Journal Of Pathology and Microbiology .2008; 51(2):190-94.

4) Kipp BR, Medeiros F, Campion MB, Distad TJ, Peterson LM, Keeney GL et al."Direct Uterine Sampling with the Tao Brush Sampler Using a Liquid-Based Preparation Method for the Detection of Endometrial Cancer and Atypical Hyperplasia: A Feasibility Study," Cancer 2008 ;114:228-35.

5) Papaefthimiou M, Symiakaki H, Mentzelopoulou P, Tsiveleka A, Kyroudes A, Voulgaris Z et al. "Study on the Morphology and Reproducibility of the Diagnosis of Endometrial Lesions Utilizing Liquid-Based Cytology" .Cancer 2005;105: 56-64.

6) Thrall M, Kjeldahl K Gulbahce HE and Pambuccian SE. “Liquid-Based Papanicolaou Test (SurePath) Interpretations before Histologic Diagnosis of Endometrial Hyperplasias and Carcinomas: Study of 272 Cases Classified by the 2001 Bethesda System” .Cancer 2007;111: 217-23

7) Watanabe J, Nishimura Y, Tsunoda S, Kawaguchi M, Okayasu I and Kuramoto H. “Liquid-Based Preparation for Endometrial Cytology-Usefulness for Predicting the Prognosis of Endometrial Carcinoma Preoperatively”. Cancer 2009;117:254-63.

8) Komatsu K, Nakanishi Y, Seki T, Yoshino A, Fuchinoue F, Amano S et al. “Application of Liquid-Based Preparation to Fine Needle Aspiration Cytology in Breast Cancer” .Acta Cytologica 2008;52: 591-96.

9) Sartelet H, Lagonotte E,. Lorenzato M, Duval I, Lechki C,.Rigaud G et al. “Comparison of Liquid Based Cytology and Histology for the Evaluation of HER-2 Status Using Immunostaining and CISH in Breast Carcinoma” .Journal of Clinical Pathology 2005;58: 864-71.

10) Fadda G and Rossi ED. “Liquid-Based Cytology in Fine-Needle Aspiration Biopsies of the Thyroid Gland,” Acta Cytologica 2011;55: 389-400.

11) Hashimoto K, Morimoto A, Kato M, Tominaga Y, Maeda N, Tsuzuki T et al. “Immunocytochemical Analysis for Differential Diagnosis of Thyroid Lesions Using Liquid-Based Cytology”. Nagoya Journal of Medical Science 2011;73:15-24.

12) Gabriel C, Achten R and Drijkoningen M.“Use of Liquid-Based Cytology in Serous Fluids: A Comparison with Conventional Cytopreparatory Techniques” .Acta Cytologica 2004;48: 825-35.

13) Laucirica R, Bentz JS, Souers RJ, Wasserman PG, Crothers BA, Clayton AC et al. “Do Liquid-Based Preparations of Urinary Cytology Perform Differently than Classically Prepared Cases? Observations from the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology” .Archives of Pathology & Laboratory Medicine 2010;134:19-22.

14) Norimatsu Y, Kawanishi N, Shigematsu Y, Kawabe T, Ohsaki H and Kobayashi TH. “Use of Liquid-Based Preparations in Urine Cytology: An Evaluation of LiquiPREP and BD SurePath” .Diagnostic Cytopathology 2010;38: 702-04

15) PiatonE, Hutin K, Faynel J, Ranchin MC and Cottier M.“Cost Efficiency Analysis of Modern Cytocentrifugation Methods Versus Liquid Based (Cytyc Thinprep) Processing of Urinary Samples”. Journal of Clinical Pathology 2004;57:1208-12.

16) Deshpande V and McKee GT. “Analysis of Atypical Urine Cytology in a Tertiary Care Center,” .Cancer 2005;105:468-75

17) Hwang EC, Park SH, Jung SI, Kwon DD, Park K, Ryu SB et al. “Usefulness of Liquid-Based Preparation in Urine Cytology”. International Journal of Urology 2007;14: 626-29.

18) Piaton E, Faynel J, Ruffion A, Lopez JG, Perrin P and Devonec M. “p53 Immunodetection of Liquid-Based Processed Urinary Samples Helps to Identify Bladder Tumours with a Higher Risk of Progression”. British Journal of Cancer 2005;93:242-47.

19) Voss JS, Kipp BR, Krueger AK, Clayton AC, Halling KC, Karnes RJ et al.“Changes in Specimen Preparation Method May Impact Urine Cytologic Evaluation”. American Journal of Clinical Pathology 2008;130: 428-33

20) Sioutopoulou DO,.Kampas LI, Gerasimidou D, Valeri RM, Boukovinas I, Tsavdaridis D et al.“Diagnosis of Metastatic Tumors in Cerebrospinal Fluid Samples Using Thin-Layer Cytology”. Acta Cytologica 2008;52 : 304-08.

21) Tanaka T, Kanda T, Sakaguchi S, Munakata S and Ohmichi M. “Vaginal Stump Metastasis from Sigmoid Colon Cancer”. Acta Cytologica 2012,56:92-96

22) Johnston WW. The malignant Pleural Effusion, a review of cytopathologic diagnoses of 584 specimens from 472 consecutive patients. Cancer 1985; 56: 905-09.

23) McGrew EA. The role of exfoliative cytology in the diagnosis and treatment of malignant disease. Postgraduate Medical Journal 1961; 37: 456-66.

24) Grey W and Mckee G. Diagnostic Cytopathology. 2nd edition.Philadelphia:Elsevier Science Limited; 2003: pp471.

25) Keettel WC,Elkins HB.Experience with radioactive colloidal gold in the treatment of ovarian carcinoma.American Journal Of Obstretics and Gynecology 1956;71:553-68.

26) Gia-Khanh Nguyen. Urine in Urinary Tract Lesions.In: Nguyen GK. Essentials of fluid cytology. Canada: Library and Archives; 2010.pp83

27) Carnoy JB. Some observations on cell division in animals. Cell.1887, 3: 225-324.

28) Pundel JP, Ferriera CDA, Peters H, deBrux J, Nieburgs HE, Schueller E, et al. II. Experiences with various methods of fi xation of smears. Do cellular changes occur as a result of air drying of smears?. Acta Cytologica 1957;1(1):62–9.

29) Seal SH. A sieve for the isolation of cancer cells and other large cells from the blood. Cancer 1964;17(5):637–42.

30) Gary Gill W. Specimen Collection. In: Dorothy Reosenthal (ed) Cytopreparation Principles and practice. New York : Springer; 2013.pp25

31) Adrian Warfield T.Cytopathology.In:John Crocker and David Burnett (eds).The science of laboratory diagnosis. second edition. England :John Wiley & sons Ltd;2005.pp105-06(a),pp95-7(b)

32) Heffner J, Brown L, Barbieri C . "Diagnostic value of tests that discriminate between exudative and transudative pleural effusions. Primary Study Investigators". *Chest* 1997;111 (4): 970–80

33) Tao.Liang-Che.Cytopathology of malignant effusions.United States: American Society of Clinical Pathologists Press;1996.

34) VicidominiG,Santini M,Fiorello A,Parascandolo V, Calabro B,Pastore V.Intraooperative pleural lavage:is it a valid prognostic factor in lung cancer?Annals of Thoracic Surgery 2005;79:254-57.

35) Enastu S, Yoshida J, Yokose T,et al. Pleural lavage cytology before and after lung resection in non-small cell lung cancer patients. Annals of Thoracic Surgery 2006;81:298-304.

36) Mohamed KH, Mobasher AA, Yousef AI et al. pleural lavage; a novel diagnostic approach for diagnosing exudative pleural effusion. Lung 2000;178:371-79.

37) Burh J,Berghauser KH,Morr H,Dobroschke J,Ebner HJ.Tumoue cells in intraoperative pleural lavage.An indicator for the poor prognosis of bronchogenic carcinoma.Cancer 1990;65:1801-04.

38) Jiao X,Zhang M,Wen Z,Krasna MJ.Pleural lavage cytology in esophageal cancer without pleural effusions: clinicopathologic analysis.European Journal Cardio-thoracic Surgery 2000;17:575-79.

39) Khan N, Sherwani K R,Afroz N,Kapoor S.Usefulness of cell block versus smears in malignant effusion cases.Journal of cytology 2006; 23:129-32.

40) Working party. Histopathology and Cytopathology Of limited or No Clinical Value. The Royal College of Pathologists;2005.

41) Selvaggi SM,Migdal S.Cytologic features of atypical mesothelial cells in peritoneal dialysis fluid. Diagnostic Cytopathology 1990;6:22-26

42) Geisinger KR, Ng LW, Hopkins MB,et al.Tenckhoff catheter cytology in patients with ovarian cancer. Cancer 1988;62(8) 1582-85.

43) Fedare O, Mariappan MR, Hileeto D, et al.Upstagimg based solely on positive peritoneal washing does not affect outcome in endometrial cancer. Modern Pathology 2005;18(5):673-80.

44) Wong FC, Pang CP, Tang SK et al.Treatment results of endometrial carcinoma with positive peritoneal washing,adnexal involvement and serosal involvement .Clinical Oncology 2004;16(5):350-55.

45) Ito K, Noda K.Peritoneal cytology in patients with uterine cervical carcinoma.Gynecol Onco.1992; 47(1):76-79.

46) De Manzoni G,Di Leo A, Tomezzoli A et al.Prognostic value of peritoneal lavage cytology in gastric cancer.Chir Ital 2002;54 (1):1-6

47) Yachida S,Fukushima N,Sakamoto M, et al. Implication of peritoneal washing cytology in patient with potentially. British Journal Of Surgery 2002;89(5):573-578

48) Chandra A, Cross P, Denton K, Giles T, Hemming D, Paynet C et al. The BSCC Code of Practice – exfoliative cytopathology (excluding gynaecological cytopathology). Cytopathology 2009;20: 211-23.

49) Allen DJ, Challacombe B,Clovis JS et al.Urine cytology: appropriate usage maximizes sensitivity and reduces cost. Cytopathology 2005;16:139-42

50) McClatchey KD, Dhurander N, Gallo L, Gill GW, Kurtycz DFI, Plowden K, Radcliffe, Trew C. Nongynecologic Cytologic specimens: collection and cytopreparatory techniques; approved guideline. NCCLS document GP23-A. NCCLS, USA; 1999.

51) Roffe BD, Wagner Fh,Derewicz HJ, Gill GW.Heparinised bottles for the collection of body cavity fluids in cytopathology.American Journal Of Hospital Pharmacy 1979;36:211-214.

52) Broghmer WL Jr ,Richardson ME,Faurest SE. Malignancy-associated serosanginous pleural effusions.Acta Cytologica 1984;28:46-50.

53) Naylor B.Novak PM. Charcol Leyden crystals in pleural fluids.Acta Cytologica 1985;29:781-784.

54) Baig MA, Fathallah L, Feng J, Husain M, Grignon DG, Al-Abbadi MA. Fast drying of Fine Needle Aspiration slides

using a hand held fan : Impact on turn around time and staining quality. CytoJournal 2006;3:12

55) Shhidham V,Kampalath B, England J.Routine air drying of all the smears prepared during finre needle aspiration and intraoperative cytology studies: an opportunity to practice a unified protocol, offering the flexibility of choosing a variety of stining methods. Acta Cytologica 2001;45:60-68.

56) Steven A Hecht, Matthew McCormack .Comparison of three cell block techniques for detection of low frequency abnormal cells. Pathology and Laboratory Medicine International 2013;5 :1–7

57) Nithyananda A. Nathan, CFIAC, Eddie Narayan, BApp Sci, Mary M. Smith, BS, and Murray J. Horn, MApp Sci. Cell Block Cytology Improved Preparation and Its Efficacy in Diagnostic Cytology. American Journal Of Clinical Pathology 2000;114:599-606.

58) Yukihiro Kobayashi , Takeshi Uehara ,Hiroyoshi Ota . Liquid-Based Thin-Layer Cytology Can Be Routinely Used in Samples Obtained via FiberopticBronchoscope. Acta Cytologica 2011;55:69–78

59) Ji Hae Koo, Ho-chang Lee, Hyung Geun Song, Hye-Suk Han,Ki Hyeong Lee, Kang Hyeon Choe et al. Comparison of Cytologic Evaluation between Conventional Method and CellprepPius® Liquid-Based Cytology in Body Fluid. *The Korean Journal of Pathology 2009; 43: 448-52*

60) Tarik M. Elsheik,Joseph L Kirkpatrick, Howard.Comparison of Thin prep and cytospinpreparationsin the evaluation of Exfoliative cytology specimens.Cancer Cytopatholgy 2006 ; 108:144-49.

61) Bong Kyung Shin, Young Suk Lee, HoiseenJoeng , Sang HoLee,HyunchulkimAree Kim and Han KyoemKim.Detecting malignant urothelial cells by morphometric analysis of Thin Prep Liquid based Urine Cytology Specimens.Korean Journal of Cytopathology 2008; 19(2);136-43.

62) NasarYousufAlwahaibi, Nasra Said Alnoumani ,Usha Rani Bai. Comparison of ThinPrep® and ConventionalPreparations for Peritoneal and Pleural Cytology Smears. Annual Research & Review in Biology 2014 ;4(20): 3139-49.

63) Babloyan S, Voulgaris Z, Papaefthimiou M, Beglaryan G, Kyroudi A, Karakitsos P. Comparison of quality between

conventional and thinprep cytology in investigation of patients with epithelial ovarian cancer. The New Armenian Medical Journal 2009;3(3):22 – 28

64) Park JM, Jang JW, Lim SY, Lee JG, Suh IS. Evaluation of the manual method of liquid-based uterine cervicovaginalcytology - by the manual method based on SurePathTMmethodology.Korean Journal of Cytopathology 2004;15:86-91.

65) Colgan TJ, McLachlin CM, Cotterchio M, Howlett R, Seidenfeld AM, Mai VM. Results of the implementation of liquid-based cytology-SurePath in the Ontario screening program. Cancer 2004;102:362-7.

66) Fremont-Smith M, Marino J, Griffin B, Spencer L, BolickD.Comparison of the SurePath liquid-based Papanicolaousmear with the conventional Papanicolaou smear in a multisite direct-to-vial study.Cancer 2004;102:269-79.

67) Zendehrokh N, Westman A,. Bjelkenkrant CH, Dejmek A.LIQUID BASED CYTOLOGY (SUREPATH/CRR) OF URINE – DIAGNOSTIC ACCURACY AND PITFALLS. Eslöv 2009; 22-23.

68) Zardawi IM, Duncan J. Evaluation of a centrifuge method and thin-layer preparation in urine cytology. Acta Cytologica 2003;47(6):1038-42.

69) Hao Deshou, Wang Changhua, Li Qinyan, Liu Wei, and Fu Wen.Clinical utility of Liqui-PREP™ cytology system for primary cervical cancer screening in a large urban hospital setting in China. Journal of Cytology 2009; 26(1): 20–25.

70) Jongkolnee Settakorn, Samreung Rangdaeng, Natchanun Preechapornkul, Sudaporn Nateewatana, Kampol Pongsiralai, Jatupol Srisomboon et al.Interobserver Reproducibility with LiquiPrepTM Liquid-based Cervical Cytology Screening in a Developing Country. Asian Pacific Journal of Cancer Prevention 2008;9:92-96

71) Roghaei MA, Afshar Moghaddam N, Sh Pooladkhan, Sh Roghaie .Adequacy criteria and cytomorphological changes in liqui-prep TM versus conventional cervical cytology. Shiraz E Medical Journal 2010;11(4): 173-82.

72) Tunc Canda,Namik Demir, Orcun Sezer, Latife Doganay, Ragip Ortac. Liqui-PREPTM, in Comparison with Conventional Smears for Detection of Squamous Cell

Abnormalities. Asian Pacific journal of cancer prevention 2009;10(3):399-402

73) Gyeongsin Park, M.D., Kyungji Lee,Chan-Kwon Jung, Dae-Hyoung Lee, Bin Cho, Youn-Soo Lee, Sang-In Shim, Kyo-Young Lee, Chang-Suk Kang. Evaluation for Cytopreservability ofManual Liquid-Based Cytology Liqui-PREPTM and its Application to Cerebrospinal Fluid Cytology: Comparative Study with Cytospin. Korean Journal of Cytopathology 2007;18(1): 46-54

74) Norimatsu et al. Use of Liquid-Based Preparations in Urine Cytology: An Evaluation of Liqui-Prep™ and BD SurePath™. Diagnostic Cytopathology 2009;38(9):702-04.

75) Seung –Myoung Son et al. Evaluation of Urine Cytology in Urothelial Carcinoma Patients: A Comparison of CellprepPlus® Liquid-Based Cytology and Conventional Smear.Korean Journal of Pathology 2012; 46(1): 68–74.

76) Parker J and Philippe E. Current and Emerging Bladder Cancer Urinary Biomarkers. The Scientific World Journal 2011; 11: 1103-12.

77) Johnston WW. The malignant Pleural Effusion, a review of cytopathologic diagnoses of 584 specimens from 472 consecutive patients. Cancer 1985; 56: 905-09.

78) Koss LG, Deitch D, Ramanathan R. Diagnostic value of cytology of voided urine. Acta Cytologica 1985; 29: 810-816.

79) Venrick MG, Sidawy MK. Cytologic evaluation of serous effusions. Processing techniques and optimal number of smears for routine preparation. American Journal of Clinical Pathology 1993; 99(2): 182-6.

80) Kumar ND, Bhatia A, Misra K, Suri JC. Comparison of pleural fluid cytology and pleural biopsy in the evaluation of pleural effusion. Journal of Indian Medical Association 1995; 93(8): 307-9.

81) Ong KC, Indumati V, Poh WT, Ong YY. The Diagnostic Yield of pleural fluid cytology in malignant pleural effusions. Singapore Medical Journal 2000; 41: 1-8.

82) Bjelakovic G, Tasic T, Stamenkovic, Stojkovic M, Katic V, Ostasinovic M et al. Biochemical, Cytological and Microbiological characteristics of the cirrhotic, malignant and “mixed” ascites. Archive of oncology 2001;9:95-101.

83) Yamamoto S, Akasu T, Fujita S, Moriya Y. Long term Prognostic value of conventional peritoneal Cytology after curative Resection for colorectal Carcinoma. Japanese Journal of Clinical Oncology 2003; 33: 33-37.

84) Jha R, Shrestha HG, Sayami G, Pradhan SB. Study of effusion cytology in patients with simultaneous malignancy and ascites. Kathmandu University Medical Journal 2006; 4 (16): 483-87.

85) Archana Joshi, Nidhi Mahajan, Karmarkar. Diagnostic utility of various techniques used in body fluid cytology. IOSR Journal of Dental and Medical Sciences 2014;13(1):13-18.

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Comparison between liquid based Cytology and Conventional cytoprepatory techniques in Body cavity fluids.

Principal Investigator : Dr. P.U Swathy

Designation : PG in MD (Pathology)

Department : Department of Pathology
Government Stanley Medical College,
Chennai-01

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 08.11.2013 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

KVasantha

MEMBER SECRETARY,
IEC, SMC, CHENNAI

சுய ஒப்புதல் படிவம்

பெருங்குடல் பற்றி ஒரு ஆய்வு

ஆராய்ச்சி நிலயம் : நோய்க் குறியியல்த் துறை

ஸ்ட்டான்லீ மருத்துவ கல்லூரி ,

சென்னை- 600001

பங்கு பெருபவரின் பெயர் :

பங்கு பெருபவரின் எண் :

மருத்துவம் ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டுள்ளது . எனது பெருங்குடல் சதை ஆய்வு பற்றிய சந்தேகங்களை கேட்கவும் அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்க பட்டது.

நான் எனது சதையை இவ்வாய்வில் பயன்படுத்த தான் இச்சையாக சம்மதிக்கிறேன் . எக்காரணத்தாலும் எந்த கட்டத்திலும் எந்த சாட்சிகளுக்கும் உட்படாமல் நான் இவாய்வில் விலகிக்கொள்ளலாம் என்றும் அறிந்து கொண்டேன்.

இந்த ஆய்வு மூலம் கிடைக்கும் தகவல்களையும் பரிசோதனை முடிவுகளையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன் படுத்திகொள்ளவும் அதை பிறசுரிக்க்வும் நான் முழு மனதுடன் சம்மதிக்கிறேன். எனக்கு கொடுக்கப்பட்ட அறிவுரைகளின் படி நடந்து கொள்வதுடன் இந்த ஆய்வு மேற்கொள்ளும் மருத்துவருக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதி அளிக்கிறேன்.

பங்கு பெறுபவரின் கைஒப்பம் : இடம் :

நாள் :

கட்டை விரல் ஒப்பம் :

பங்கு பெறுபவரின் பெயர் மட்டும் விலாசம் :

தகவல் படிவம்

தங்களுக்கு செய்த உடல் திரவங்கள் ஆராய்ச்சி மூலம் தங்களுக்கு பிரச்சனை உள்ளது கண்டுபிடிக்கப்பட்டுள்ளது. அதற்கான காரணம் அறிய ஆய்வு மேற்கோள்ளப்பட உள்ளது. இதில் தங்களது நோய் குறித்து விவரங்கள் இதர உடல் திரவங்கள் ஆராய்ச்சி முடிவுகளை தங்கள் சம்மதத்துடன் இவ்வாய்வில் பயன்படுத்த விரும்புகிறோம். பின்னாளில் மீண்டும் ஆய்வில் பங்கேர்க்கவும் தங்கள் இவ்வாய்வில் தங்கள் முழு சம்மதம் பெற்ற பின்னர் மட்டும் மேற்கோள்ளப்படும்.

தங்கள் விரும்பினால் இவ்வாய்வில் இருந்து எப்பொழுது வேண்டுமானாலும் எந்த சாட்சிகளுக்கும் உட்படலம் , விலகிக்கொள்ளலாம்.

இவ்வாய்வில் மூலம் கிடைக்கும் தகவல்களும், பரிசோதனை முடிவுகளும் தங்களின் ஒப்புதல் மூலம் மட்டுமே ஆய்வில் பயன்படுத்தப் படும்.

ஆய்வாளரின் கைஒப்பம் :

இடம்

நாள

ஆய்வளரின் பெயர் :

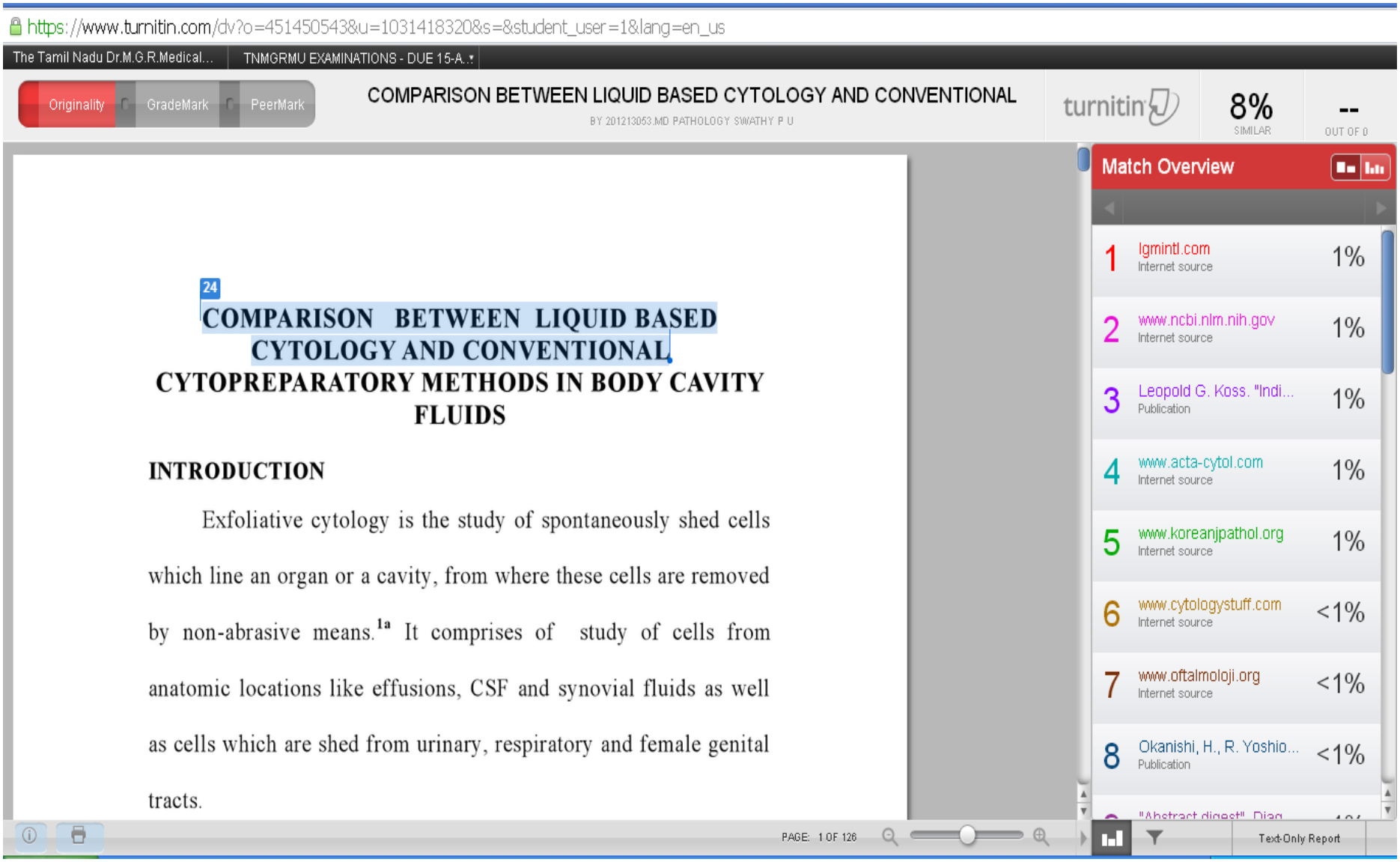
S. No	Cytology Number	Age	Sex	Type of Fluid	Cellularity grade		Uniform cell distribution		Cell morphology		Background	
					CS	LBC	CS	LBC	CS	LBC	CS	LBC
1	2250/12	50	F	peritoneal	1	1	2	1	1	1	1	1
2	2333/12	39	M	peritoneal	1	2	1	2	1	2	1	2
3	2450/12	66	M	pleural	1	1	1	1	1	2	1	1
4	2462/12	75	M	peritoneal	1	1	2	2	1	2	1	2
5	2555/12	55	M	peritoneal	1	2	2	2	2	2	1	1
6	2657/12	55	M	urine	0	1	0	1	0	0	0	1
7	2684/12	45	F	peritoneal	1	1	1	1	1	2	1	1
8	2893/12	13	M	pleural	1	2	1	2	2	2	1	2
9	2908/12	46	M	peritoneal	0	0	0	0	0	0	0	0
10	3045/12	65	M	pleural	2	2	1	2	1	2	1	1
11	3071/12	35	F	peritoneal	1	1	2	1	2	1	1	1
12	3187/12	55	F	peritoneal	1	2	1	2	1	2	1	1
13	3216/12	50	M	peritoneal	1	2	0	2	1	2	1	1
14	3348/12	60	M	pleural	1	2	1	1	1	2	0	1
15	3397/12	52	M	peritoneal	1	1	0	1	1	2	1	1
16	3467/12	45	M	peritoneal	2	1	2	0	2	1	2	1
17	3520/12	21	M	urine	1	2	1	2	1	2	1	1
18	3602/12	46	F	peritoneal	0	0	1	1	0	0	0	0
19	3748/12	48	M	peritoneal	1	1	0	2	1	2	1	1
20	3921/12	25	M	pleural	1	1	1	2	2	2	2	2
21	4041/12	50	F	urine	0	1	1	1	0	1	1	2
22	4176/12	71	M	peritoneal	1	2	2	2	2	1	2	2
23	4222/12	68	M	pleural	1	2	0	0	1	2	0	1
24	4465/12	52	M	peritoneal	1	2	1	2	1	2	1	1
25	4526/12	40	M	peritoneal	1	2	1	2	1	2	1	2
26	4600/12	65	F	peritoneal	2	2	1	2	2	2	2	1
27	4745/12	80	M	pleural	2	2	0	1	1	2	2	1
28	4756/12	70	M	urine	1	1	1	2	1	1	1	2

S. No	Cytology Number	Age	Sex	Type of Fluid	Cellularity grade		Uniform cell distribution		Cell morphology		Background	
					CS	LBC	CS	LBC	CS	LBC	CS	LBC
29	4762/12	35	M	peritoneal	2	2	2	2	2	2	1	1
30	211/13	47	F	peritoneal	1	1	1	1	0	1	0	0
31	347/13	75	M	peritoneal	1	2	1	2	1	2	1	1
32	398/13	36	F	pleural	1	1	0	2	2	2	1	1
33	445/13	70	M	urine	1	1	1	1	1	2	1	1
34	588/13	32	M	peritoneal	1	1	0	1	1	2	1	2
35	678/13	65	F	pleural	1	2	2	2	2	2	1	2
36	829/13	43	M	peritoneal	2	1	0	0	0	1	0	0
37	997/13	30	M	pleural	2	2	2	2	2	2	2	2
38	1342/13	30	M	peritoneal	1	1	0	1	1	2	1	1
39	1429/13	65	M	urine	0	1	0	1	0	1	1	1
40	1675/13	30	F	peritoneal	1	2	1	2	1	2	1	1
41	1756/13	19	F	pleural	1	1	1	2	2	2	1	1
42	1841/13	30	F	peritoneal	1	2	1	2	1	2	1	1
43	1934/13	75	M	pleural	2	1	1	2	1	1	1	1
44	2244/13	53	M	pleural	1	1	0	1	1	1	1	1
45	2314/13	45	M	urine	2	2	1	2	1	1	2	1
46	2567/13	40	F	pleural	1	2	1	2	1	2	1	1
47	2660/13	45	M	peritoneal	2	2	2	2	2	1	2	1
48	2761/13	66	M	peritoneal	1	2	1	2	1	2	1	1
49	2789/13	49	M	pleural	1	2	2	2	1	2	1	1
50	2918/13	70	M	peritoneal	1	1	1	2	1	2	2	2
51	3020/13	44	M	peritoneal	1	1	0	1	1	2	1	1
52	3163/13	38	M	peritoneal	1	2	1	2	1	2	1	1
53	3248/13	73	F	peritoneal	1	1	1	1	1	2	1	1
54	3300/13	16	M	pleural	0	0	0	0	0	0	0	0
55	3481/13	48	M	peritoneal	2	1	2	1	2	1	2	2
56	3523/13	40	M	pleural	1	2	1	2	1	2	2	2

S. No	Cytology Number	Age	Sex	Type of Fluid	Cellularity grade		Uniform cell distribution		Cell morphology		Background	
					CS	LBC	CS	LBC	CS	LBC	CS	LBC
57	3649/13	43	M	peritoneal	2	1	1	2	1	2	2	1
58	3745/13	35	M	pleural	1	1	1	2	1	2	2	2
59	3811/13	46	M	pleural	2	2	2	2	1	1	1	1
60	3976/13	49	M	peritoneal	1	1	1	1	1	1	1	1
61	4057/13	65	F	peritoneal	1	2	1	2	1	1	1	1
62	4152/13	44	M	pleural	1	2	1	1	1	2	1	1
63	4375/13	46	M	pleural	1	1	1	2	1	2	1	2
64	4465/13	44	F	peritoneal	0	0	2	1	0	0	0	0
65	4690/13	58	M	peritoneal	1	2	1	2	1	2	1	1
66	4765/13	33	M	pleural	2	2	2	2	2	2	2	1
67	25/14	42	F	peritoneal	1	1	2	0	2	2	1	1
68	26/14	45	M	pleural	1	1	1	2	1	1	1	1
69	29/14	70	M	peritoneal	1	1	1	1	1	2	1	1
70	655/14	43	M	urine	0	1	1	1	0	2	0	1
71	740/14	33	M	pleural	1	2	1	2	2	2	1	2
72	770/14	52	M	peritoneal	1	2	1	2	1	2	1	1
73	793/14	58	F	peritoneal	1	1	1	1	1	2	1	1
74	811/14	36	M	pleural	1	1	1	0	1	2	0	0
75	812/14	50	M	peritoneal	1	2	1	2	2	2	1	1
76	823/14	40	M	peritoneal	1	1	1	1	1	2	1	1
77	838/14	17	M	pleural	1	2	1	2	2	2	2	2
78	867/14	27	M	pleural	1	1	1	2	2	2	1	2
79	879/14	38	M	peritoneal	1	2	1	1	1	1	1	1
80	913/14	56	M	peritoneal	2	2	2	2	1	1	2	1
81	970/14	55	M	pleural	0	0	0	1	1	1	0	0
82	1137/14	47	M	pleural	1	2	1	2	1	1	2	1
83	1153/14	35	M	pleural	1	1	1	1	1	2	1	1
84	1217/14	43	F	peritoneal	2	2	1	2	1	2	0	1

S. No	Cytology Number	Age	Sex	Type of Fluid	Cellularity grade		Uniform cell distribution		Cell morphology		Background	
					CS	LBC	CS	LBC	CS	LBC	CS	LBC
85	1226/14	39	M	peritoneal	1	1	1	2	2	2	1	1
86	1400/14	55	M	urine	0	1	0	1	0	1	1	1
87	1429/14	60	F	peritoneal	2	1	1	2	2	2	1	2
88	1435/14	46	M	pleural	1	1	1	2	1	2	1	1
89	1484/14	55	M	peritoneal	1	2	1	2	2	2	1	2
90	1514/14	60	M	peritoneal	1	2	1	2	1	2	1	2
91	1586/14	63	M	peritoneal	1	1	1	1	1	2	1	1
92	1598/14	57	M	pleural	2	2	1	2	2	2	1	1
93	1601/14	60	M	urine	2	2	2	2	2	2	2	2
94	1619/14	37	M	peritoneal	1	1	1	1	1	2	1	1
95	1624/14	70	F	peritoneal	1	1	0	2	1	2	1	2
96	1631/14	56	M	pleural	1	1	1	2	1	2	2	2
97	1707/14	51	M	peritoneal	2	1	1	1	1	2	1	1
98	1709/14	62	M	urine	1	1	1	1	1	1	1	1
99	1713/14	50	M	peritoneal	0	0	1	1	1	1	1	1
100	1751/14	43	F	peritoneal	0	0	1	2	1	2	1	1

0- Score 0, 1- Score 1, 2-Score 2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COMPARISON BETWEEN LIQUID BASED CYTOLOGY AND CONVENTIONAL CYTOPREPARATORY METHODS IN BODY CAVITY FLUIDS
INTRODUCTION
Exfoliative cytology is the study of spontaneously shed cells which line an organ or a cavity, from where these cells are removed by non-abrasive means.[1a] It comprises of study of cells from anatomic locations like effusions, CSF and synovial fluids as well as cells which are shed from urinary, respiratory and female genital tracts.
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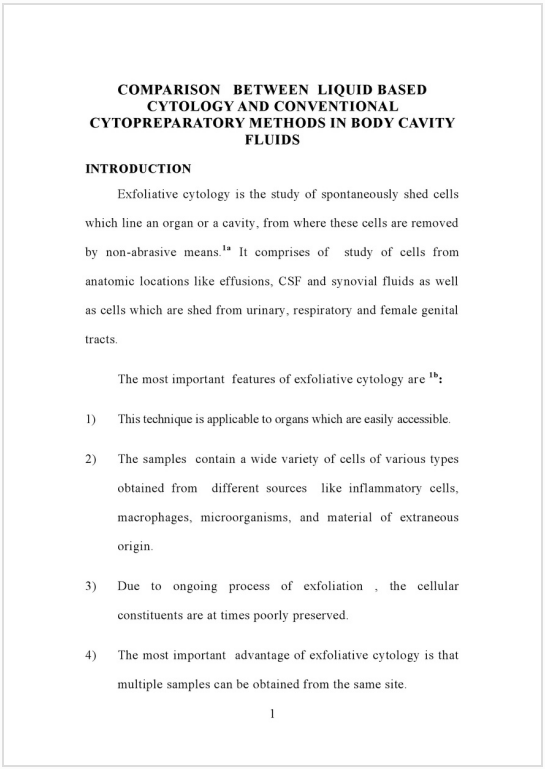

COMPARISON BETWEEN LIQUID BASED CYTOLOGY AND CONVENTIONAL CYTOPREPARATORY METHODS IN BODY CAVITY FLUIDS

INTRODUCTION

Exfoliative cytology is the study of spontaneously shed cells which line an organ or a cavity, from where these cells are removed by non-abrasive means.[1a] It comprises of study of cells from anatomic locations like effusions, CSF and synovial fluids as well as cells which are shed from urinary, respiratory and female genital tracts.

The most important features of exfoliative cytology are [1b]:

1) This technique is applicable to organs which are easily accessible.
2) The samples contain a wide variety of cells of various types obtained from different sources like inflammatory cells, macrophages, microorganisms, and material of extraneous origin.
3) Due to ongoing process of exfoliation , the cellular constituents are at times poorly preserved.
4) The most important advantage of exfoliative cytology is that multiple samples can be obtained from the same site.

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